American Association for the Advancement of Science

SCIENCE

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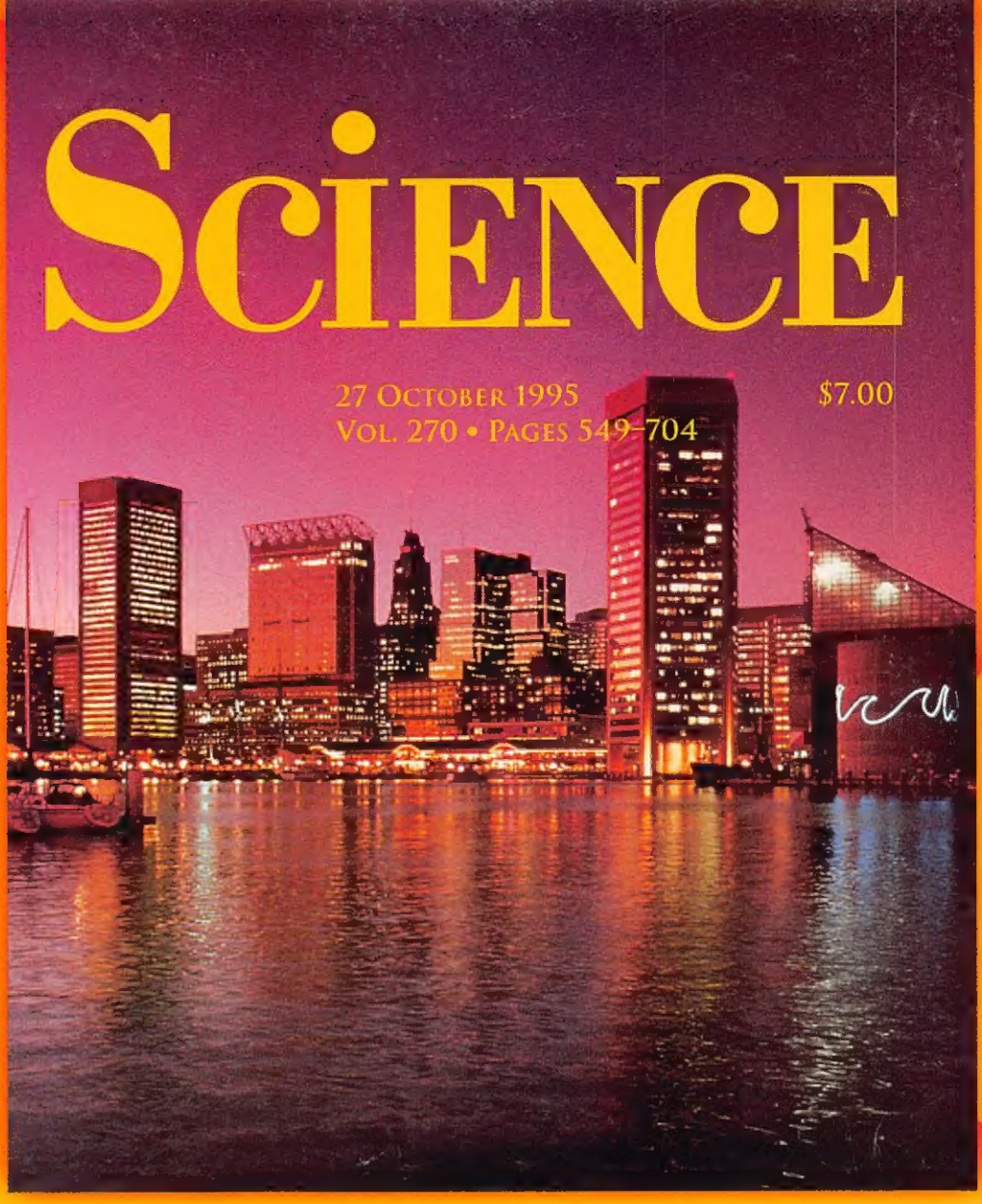

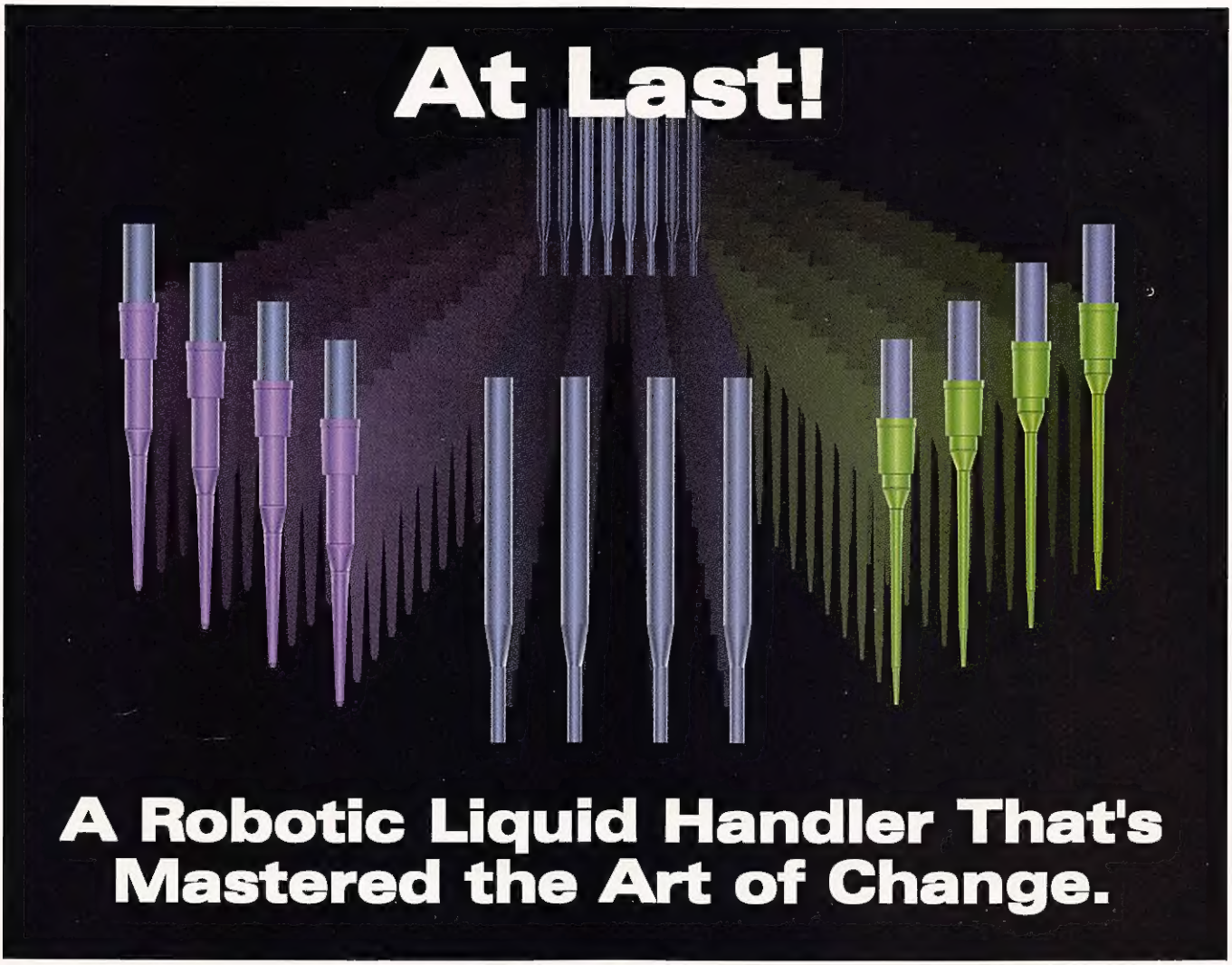

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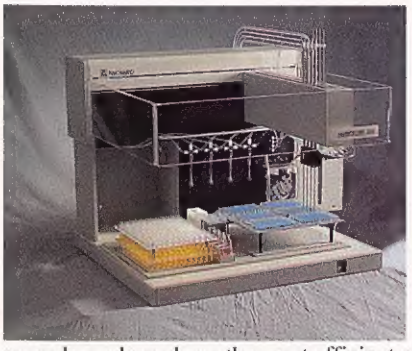

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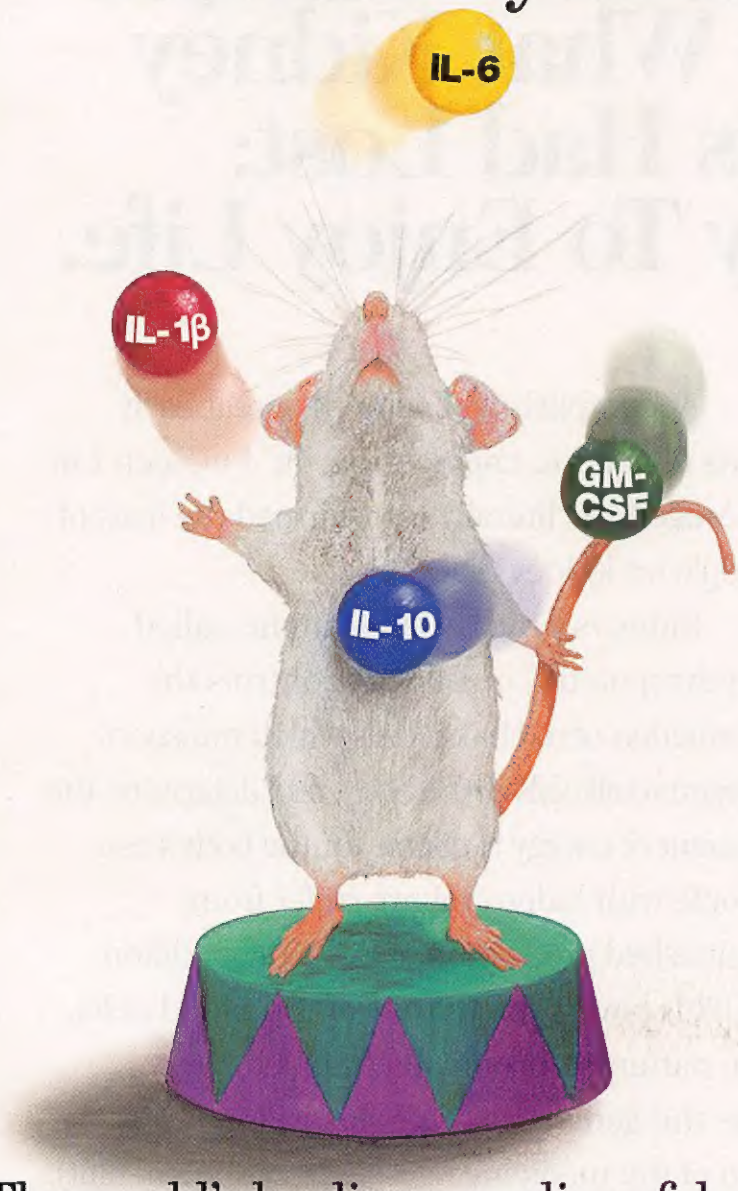

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1995 Discoverers Award

This Man's Discovery Restored What Kidney Patients Had Lost: The Energy To Enjoy Life.

Dr. Fu-Kuen Lin, Director Biomedical Sciences, Amgen

Accomplishing one of the great early feats of genetic engineering, Dr. Fu-Kuen Lin of Amgen has literally transformed the lives of people on kidney dialysis.

Kidneys produce a hormone called Erythropoietin, or EPO. It controls the production of red blood cells which transport oxygen to all cells of the body, and determine the amount of energy available for the body's use. People with kidney failure suffer from diminished production of EPO – a condition which dialysis does not correct. This lowers the output of red blood cells, reduces oxygen, and can leave patients chronically fatigued.

Dr. Lin was able to clone the gene that produces EPO, making possible the production of the medicine, EPOGEN® (Epoetin alfa).

It has vastly improved the quality of life for people on kidney dialysis – some two hundred thousand Americans. Many can now return to their daily routine and enjoy their families more actively.

For his work, Dr. Lin has been named recipient of the 1995 Discoverer's Award, which honors the outstanding contributions of scientists from America's pharmaceutical research companies.

America's Pharmaceutical Research Companies

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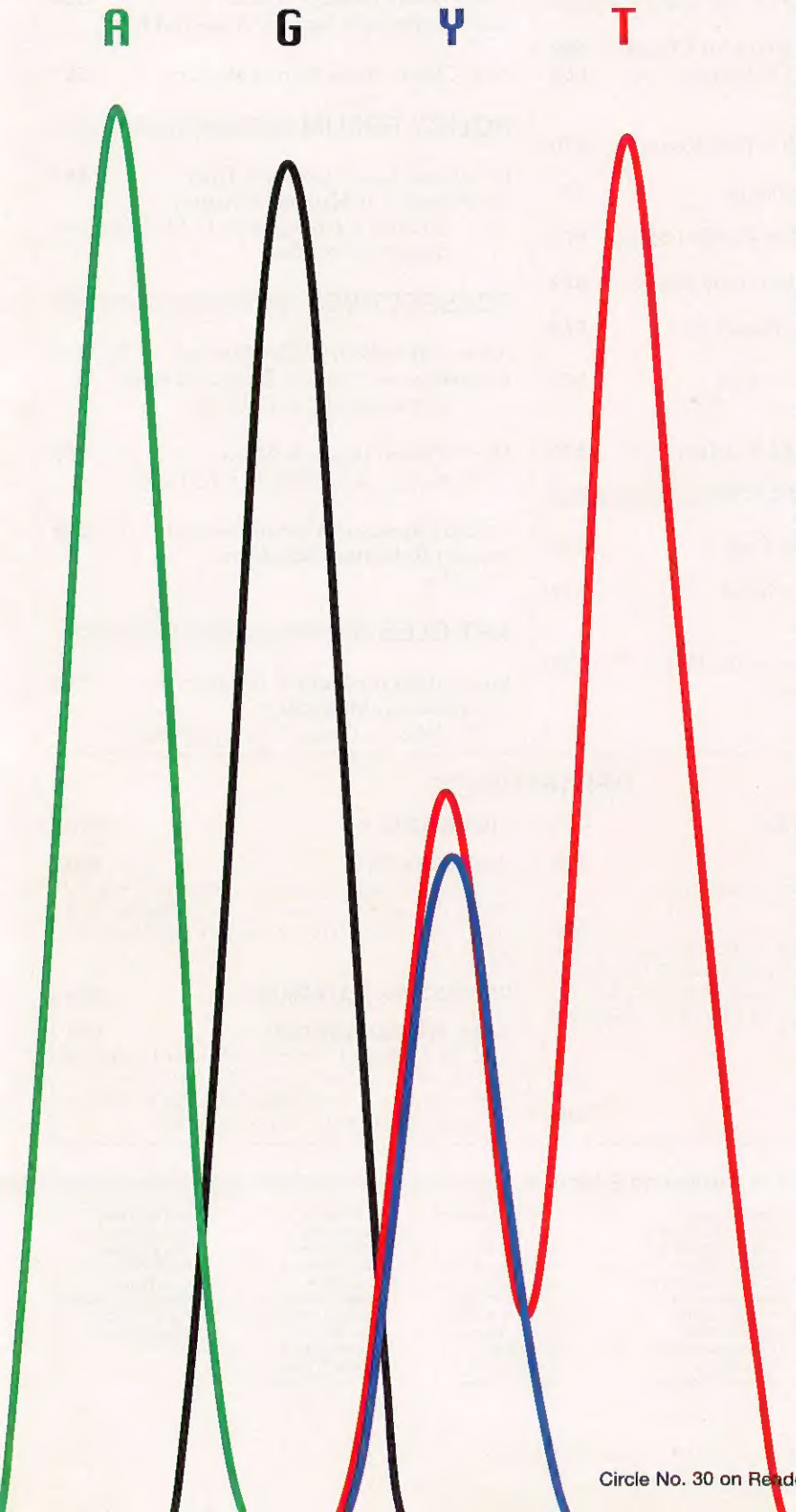
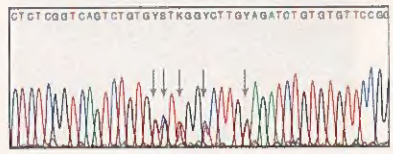
Even peaks make mutation detection even easier.



Good news for genetic disease researchers. A breakthrough in enzyme design just took mutation detection to new levels of convenience and performance.



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AmpliTaq FS dye primer kits make it easier than ever to directly sequence PCR products. They combine the simplicity of cycle sequencing with high-efficiency ddNTP incorporation to produce precise data with uniform peak heights. The result is accurate detection of mutations.

AmpliTaq FS kits expand the power of ABI PRISM™ multicolor detection technology to sequence-based mutation detection. Together with our reagents and protocols for fluorescent PCR detection, we provide complete DNA analysis solutions for your laboratory.

Find out how PCR-based cycle sequencing with AmpliTaq FS kits makes mutation detection even easier. For more information and a free copy of our technical guide to comparative PCR sequencing, call 1-800-345-5224. Outside the U.S. and Canada, contact your local Perkin-Elmer representative.

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Perkin-Elmer PCR reagents are developed and manufactured by Roche Molecular Systems, Inc., Branchburg, New Jersey, U.S.A.

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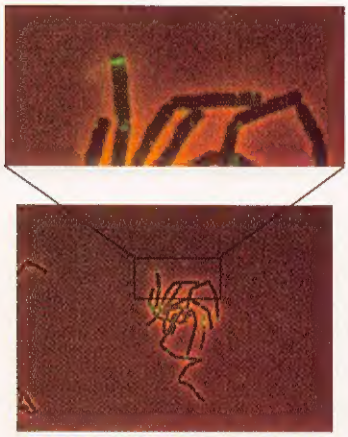
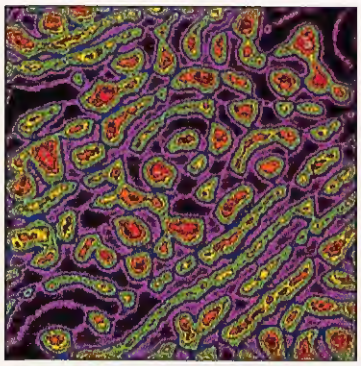


578, 637 & 641
Dividing line of cell fate



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Probing membranes with near-field optics

NEWS & COMMENT

RESEARCH NEWS

POLICY FORUM

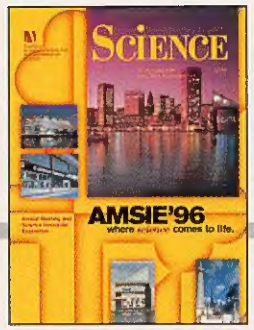
PERSPECTIVES

ARTICLES

DEPARTMENTS

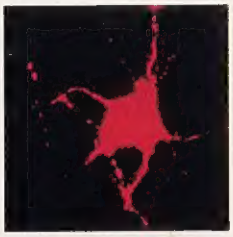
COVER

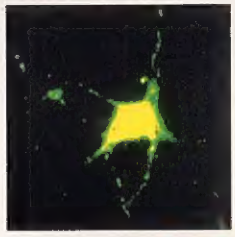
"Where Science Comes to Life" is the theme for the 1996 AAAS Annual Meeting and Science Innovation Exposition in Baltimore from 8 to 13 February. Researchers from diverse disciplines will discuss new knowledge and what is to come, not only scientifically but with regard to the relation of science and society. See page 655 for a complete program with registration and hotel forms. [Cover design: James B. Hicks III. Photos (clockwise from main image): R. Miller, M. Evans, R. Miller, BACVA, and J. Rettaliata]



RESEARCH ARTICLE

REPORTS





593

Neurotrophins on the move

AAAS Board of Directors

Indicates accompanying feature

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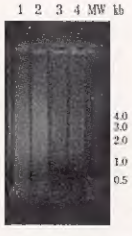
Figure 1.



mRNA isolated from 8 x 10^7 HeLa cells. Each pellet was resuspended in 50 µl RNase-free H_2O. 5 µl was loaded onto a 1% agarose gel. Lanes 1 and 2: FastTrack® 2.0 Lanes 3 and 4: Magnetic Beads

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edited by PHIL SZUROMI

Shedding light on membranes

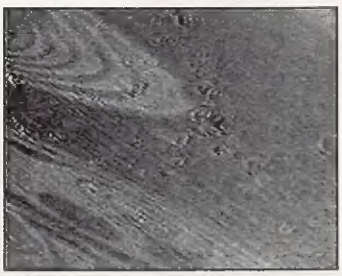
Lipid monolayers are used to construct two-dimensional arrays and as a model for biological membranes. These monolayers exhibit domain structure that can vary as a function of chemical composition, pressure, and temperature; however, information on the detailed domain structure has been difficult to obtain. Hwang *et al.* (p. 610) have used near-field scanning optical microscopy to image domain boundaries, to measure chemical composition, and to follow the partitioning of a probe material into the different phases. Such features were not visible in far-field epifluorescence microscopy.

Arc melting

Subduction of oceanic crust fuels melting in the overlying mantle, which produces volcanic arcs. In theory, the composition of volcanic rocks sampled across a magmatic arc might be expected to reflect in some way the effect of the systematic dehydration of the subducted slab on the mantle. Ryan *et al.* (p. 625) examined the variation of several elements that should reflect changes in fluid composition in lavas from the Kurile arc. Elements with strong affinities for water decreased in abundance with distance from the subduction zone.

Coming up to air

Surface reactions are important in many catalytic processes, and their kinetics are the focus of intensive research. One of the problems in these studies is that many of the techniques used require high vacuum, so reaction conditions, especially pressure,



are often unrealistic. Rotermund *et al.* (p. 608) have developed optical techniques that allow the real-time observation of reaction kinetics of carbon monoxide oxidation on a platinum surface from high vacuum to atmospheric pressures. They also observed new phenomena in pattern formation.

Mountains in high relief

What determines the relief of mountains? Locally it would seem that the intact strength of rocks allows weathering to produce steep cliffs. Schmidt and Montgomery (p. 617), however, propose that on the larger scale of mountains, topography is controlled by mountain-scale material strength and bedrock landsliding. Strength at these scales would reflect the strength of the weakest rock units. The authors compared the slope profiles in the Cascades and Santa Cruz mountains of the western United States with models of slope stability and experimental data on rock strength.

Multicellular origins

The timing of the appearance of multicellular organisms in the Precambrian is uncertain; one problem has been that fossils of these organisms, which lacked hard parts, are scarce. Shixing and Huineng (p. 620) describe fossils resembling multicellular algae from the Tuanshanzi Formation in the Jixian area, north China, dated at approximately 1700 million years ago. The fossils range up to about 1 centimeter in length.

What mother will tolerate

Because of paternal inheritance, the fetus is usually not a histocompatible match to its mother. Why, then, if they are in contact, does the immune system of the mother not attack the fetus? Tafuri *et al.* (p. 630) show that during pregnancy, maternal T cells reactive to the father's alloantigens are tolerized. This tolerance is lost after birth, and may explain why in some cases autoimmune diseases go into remission during pregnancy.

Springing forth together

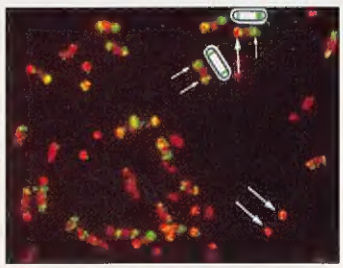
The radiation of life near the end of the Precambrian has been thought to have involved roughly an initial emergence of large soft-bodied animals in the Vendian followed by the appearance of shelly faunas in the Cambrian. Grotzinger *et al.* (p. 598; see news story by Kerr, p. 580) dated volcanic rocks interbedded with sedimentary rocks in Namibia that bracket these events. The dates and comparison of other geochemical and biostratigraphic data with other rock sections suggest that the Vendian faunas were extant from 549 to as young as 543 million years ago, which is essentially the age of the Precambrian-Cambrian boundary. There does not seem to be a great temporal hiatus between the two faunas.

Working under stress

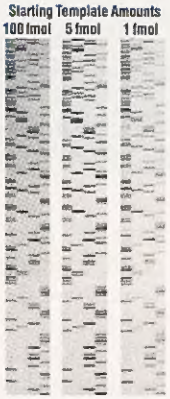
In response to stress, such as prey recognizing a predator, the sympathetic nervous system increases cardiovascular activity and releases the hormone epinephrine. Jansen *et al.* (p. 644) now show, as hypothesized by Cannon years ago, that a set of neurons controls both of these functions. Heart and adrenal gland neurons in rats were injected with two different types of attenuated virus. These viral labels could be traced back to a common set of neurons in the hypothalamus and brainstem.

Separated and unequal

How cells become specialized is a central question in developmental biology. Two reports examine the mechanism by which an asymmetric cell division in the bacteria *Bacillus subtilis* generates two progeny cells with different fates, the mother cell and the forespore (see news story



by Roush, p. 578). Arigoni *et al.* (p. 637) have studied the localization of SpoIIE, a protein required to activate spore-specific gene expression during spore formation. SpoIIE is asymmetrically localized during division at the polar septum and resides at the division site when division is complete. Duncan *et al.* (p. 641) found that SpoIIE is a serine phosphatase. Localized SpoIIE functions to overcome the inhibition of the spore-specific transcription factor σ^F.



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EDITORIAL

Strengthening Our Global Commitment

Two and one-half years ago (see *Science*, 28 June 1993) *Science* launched a campaign to expand its international operations. The intent, fully endorsed by the AAAS Board of Directors, was to reach out to readers in Europe and Asia. Feedback received from readers, reviewers, and *Science* journalists in both regions during my visits there over the past 4 months indicates that our campaign is clearly gaining momentum, as emphasized by several recent changes. Before our U.K. office in Cambridge was opened, approximately 7% of *Science* Reports had a majority of European authors. As of this month, that figure has reached 25%. Over the same period, the number of Europe- and Japan-based members of our Board of Reviewing Editors has increased from 0 out of 45 to 18 out of 74, with comparable increases in non-U.S. peer reviewers. These reasonably satisfying early results have been achieved without any geographic quotas, through fair and equal competition among submittals. Our intention is to provide a rapid and fair review system conducted by an accessible editorial staff.

Meanwhile, *Science* has also expanded its international cadre of science journalists. Two full-time European reporters are now working out of the Cambridge office and another in Tokyo, with regular correspondents in Paris, Berlin, Heidelberg, Moscow, and Beijing. The result has been well over 100 pages per year of European news coverage and about double that amount of overall non-U.S. coverage. For example, this issue examines the special research support made possible in Germany by the Max Planck Institutes (two of whose researchers won Nobel Prizes this year) and includes an interview with the newly appointed head of the Max Planck Society.

All this is, of course, just a beginning. As demonstrated in London and Paris last week to European scientists and science journalists, our World Wide Web offerings have allowed us since June 1995 to inform connected readers throughout the world of our contents on the day of publication (see *Science*, 23 June 1995). In the past 5 months, the number of unique computer addresses that visit us weekly has grown to over 8000. As other journals join in this alternative means of communication, scientists everywhere will be all the better informed.

The exploration of enhancements to our printed media is only beginning. Like other diligent editors, we have been actively soliciting reader feedback. The next version of our "*Science* On-Line" Web pages will debut shortly, offering many of the features our browsers have most often requested. We want more than simply to quell readers' curiosity until their mailed copies arrive. Our "Beyond the Printed Page" section has already shown data on genomes, computer simulations, and molecular structures that enhance what print readers can see, and our forums for the ongoing discussion of critical policy issues in science have provided an avenue for direct reader-to-reader feedback in full public view.

Speaking of our blossoming Web pages (and of science journalists), beginning today, browsers of "*Science*'s Next Wave" (http://sci.aaas.org/nextwave/) will be able to explore the pros and cons of pursuing innovative alternatives to the traditional academic research career. The first of a series of features on alternative careers focuses on science journalism. Later we will present discussions of careers in industry, patent law, science policy-making, environmental work, teaching, and many more. Like all the rest in this series, the focus on science journalism will provide both resources (descriptions of the top U.S. science journalism programs, with contact information) and role models (access to a group of carefully selected journalists and scientists, most of whom were Ph.D.'s before making the transition to journalism). Readers will be able to interact directly with these career role models at our Web site. They will tell you how they made the transition and what you will need to know to make it yourself, if you choose. This effort is designed to help our young scientists and their increasingly beleaguered advisers keep up to date on careers that do not depend on yet more government grants, by answering such questions as, What in fact are the options? How does one make the right preparations? Where are the jobs?

In the near future, both "*Science*'s Next Wave" and *Science* will be expanding their international offerings, with a special concentration on Asia. We think the need for such expansion—both internationally and into the new media of communication—is very real. Let us know your views.

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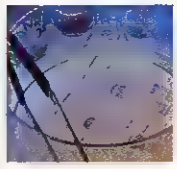

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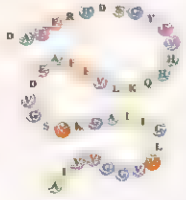

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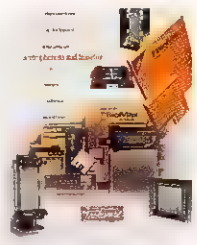

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LETTERS

One world?

Ecologists discuss a News article in our special section "Frontiers in biology: Ecology" (21 July, pp. 313–360) and subsequent letters (1 Sept., p. 1201). While 24 letter writers describe one dispute as a "minor squabble," the views expressed in other letters belie this description.

The Role of Experiments in Ecology

We thank *Science* for giving ecology coverage in the "Frontiers in Biology: Ecology" special section (21 July, pp. 313–360). It was unfortunate that the lead News article by Wade Roush, "When rigor meets reality," highlights a minor squabble that stemmed from the remarks of one postdoctoral researcher. We encourage the editors and reporters of *Science* to continue coming to ecological meetings so that they can broaden their knowledge and expand their coverage of the substantive issues. Ecology is a true frontier, being perhaps the most complex system that science has ever tried to understand. Increasingly, ecologists are combining experiments, observations, and theory to expand the temporal and spatial scale of our inferences. We are strongly motivated by the pressing need for answers to major questions of direct relevance to the long-term sustainability and habitability of Earth.

Mary E. Power, *Department of Integrative Biology, University of California, Berkeley, CA 94720, USA*; **David Tilman**, *Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, MN 55108, USA*; **Stephen R. Carpenter**, *Center for Limnology, University of Wisconsin, Madison, WI 53706, USA*; **Nancy Huntly**, *Department of Biological Sciences, Idaho State University, Pocatello, ID 83209, USA*; **Mathew Leibold**, *Department of Ecology and Evolution, University of Chicago, Chicago, IL 60637, USA*; **Peter Morin**, *Department of Biological Sciences, Rutgers University, Piscataway, NJ 08855, USA*; **Bruce A. Menge**, *Department of Zoology, Oregon State University, Corvallis, OR 97331, USA*; **James A. Estes**, *Institute of Marine Sciences, University of California, Santa Cruz, CA 95064, USA*; **Paul R. Ehrlich**, *Department of Biological Sciences, Stanford University, Stanford, CA 94305, USA*; **Mark Hixon**, *Department of Zoology, Oregon State University, Corvallis, OR 97331, USA*; **David M. Lodge**, *Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA*; **Mark A. McPeek**, *Department of Biological Sciences, Dartmouth College, Hanover, NH 03755, USA*; **John E. Fauth**, *Department of Biology, College of Charleston, Charleston, SC 29424, USA*; **David Reznick**, *Biology Department, University of California, Riverside, CA 92521, USA*; **Larry B. Crowder**, *Duke University Marine Laboratory, Beaufort, NC 28516, USA*; **Sally J. Holbrook**, *Department of Biological Sciences, University of California, Santa Barbara, CA 93106, USA*; **Barbara L. Peckarsky**, *Department of Entomology, Cornell University, Ithaca, NY 14853, USA*; **Douglas E. Gill**, *Department of Zoology, University of Maryland, College Park, MD 20742, USA*; **Janis Antonovics**, *Department of Botany, Duke University, Durham, NC 27708, USA*; **Gary A. Polis**, *Department of Biology, Vanderbilt University, Nashville, TN 37235, USA*; **David B. Wake**, *Museum of Vertebrate Zoology, University of California, Berkeley, CA 94720–3160, USA*; **Gordon Orians**, *Department of Zoology, University of Washington, Seattle, WA 98195, USA*; **Ellen D. Ketterson**, *Department of Biology, Indiana University, Bloomington, IN 47405, USA*; **Elizabeth Marschall**, *Department of Zoology, Ohio State University, Columbus, OH 43210, USA*; *and* **Sharon P. Lawler**, *Department of Entomology, University of California, Davis, CA 95161, USA.*

Roush's article portrays the American Society of Zoologists' symposium "The State of Experimental Ecology" as an "organizational rally of sorts" for the "new experimentalists" and as part of a "revisionist movement" advocating a return to more "muddy-boots biology." As co-organizer of the symposium, I strongly disagree with this portrayal. Although the coverage given to this symposium is appreciated, the article confers a negative tone on the proceedings and does not convey the scope and goals of the symposium. I also disagree with the article's presentation of the important issues in experimental ecology.

The symposium brought together experimental ecologists representing the broad array of experimental approaches used in ecology, from laboratory microcosms to manipulation of entire ecosystems, in order to illustrate the myriad ways in which experiments are applied to ecological questions. The symposium specifically emphasized the value of a plurality of experimental approaches; it was definitely *not* about attacking other ecologists or "challeng[ing] . . . colleagues' methods" (nor were my own discussions with Roush). It was experimental ecologists critiquing themselves to move experimental ecology forward on all fronts, from better designs, to better links between experiments and theory, to more realism in experiments designed to explore specific natural systems. It was also a forum in which to discuss the limitations and obstacles to applying experiments to specific ecological systems and questions. Our only agenda was to reinforce the importance of experiments and experimental rigor in un-

derstanding ecological processes and to stress the need to continually improve our application of experimental methodology and achieve better integration between experiments, theory, and natural history. Our goal was to ensure that the rate of progress in the application of experimental methods to complex ecological problems continues to accelerate. It is unfortunate that the article did not capture the energy and positive tone of the symposium, and missed the real story of experimental ecology: the tremendous progress in ecological understanding achieved through experimentation.

Similarly, the article depicts my personal views in ways that I would not and so vaguely ascribes opinions that I have subsequently been criticized, in print and elsewhere, for statements I did not make and opinions I do not hold. I presumably criticized "experiments [that] often reduce nature to oversimplified caricatures that have little to do with the real world." That certainly does not reflect my view, as much of my work makes use of mesocosms (*1*), and I firmly believe that such simplified systems instruct us about the real world. Subsequent letters (1 Sept., pp. 1201–1203) criticize me for attacking Andrew Blaustein. I was not quoted regarding his work, as I had, in fact, refused to discuss it.

The article's negative tone was amplified by exclusion of positive statements or by their paraphrasing into negative, critical statements. I have been critical (*2*) of Dolph Schluter's recent experiment (*3*) and agreed to discuss it because the paper was published and criticisms rendered in *Science*. However, my repeated caveat that criticisms were limited to the specific experiment and that Schluter's other work on character displacement is compelling was not included. Even a positive prescription for experimental ecology penned (with Joseph Bernardo) at the request of *Science* was paraphrased into a series of negative statements on what experimental ecologists "fail" to do, and then linked with another quote that neither should have been made nor printed.

There was an interesting article to be written about the tremendous strides made in ecology through experimentation and the many directions experimental ecology is taking under several generations of experimental ecologists. Indeed, many of the important figures in the evolution of experimental ecology were interviewed, many more than were represented in the article. Why, then, were these strides and directions not made the focus of the article? The rationale given by *Science*'s News editors was that these topics were simply "not engaging." I disagree.

William J. Resetarits Jr.
Center for Aquatic Ecology, Illinois Natural History Survey, Champaign, IL 61820, USA

References

1. W. J. Resetarits Jr., *Ecology* **72**, 1782 (1991); *Oikos* **73**, 188 (1995).
2. J. Bernardo, W. J. Resetarits Jr., A. E. Dunham, *Science* **268**, 1065 (1995).
3. D. Schluter, *ibid.* **266**, 798 (1994).

My purpose in criticizing high-profile ecological experiments (*1*) is to stimulate reasonable debate about the fair extent of inferences that scientists make from their experimental results. This general aim is reflected in my efforts to co-organize a symposium whose goal was to offer constructive insights to improve the future practice of experimentation in ecological and evolutionary research. It is also reflected in my efforts to ensure the accuracy of *Science*'s article, which I understood was to be about the role of experiments in contemporary ecological research, the focus of the symposium. To this end, I gave Roush our symposium proposal that detailed its diverse goals and a list of names and addresses of all of the symposium participants (many of whom he interviewed). I also spent more than 6 hours in three

interviews over several weeks expanding on these themes. Roush's article inaccurately represented the symposium and the spirit of our conversations. My criticisms span a variety of issues in the use of experimentation in ecological inference, ranging from problems of confounded designs and unnatural experimental conditions (*1*), to difficulties with the choice of experimental variables and treatment levels that affect interpretation, and overgeneralization (comments I made in Roush's article). I concur with Reznick (Letters, 1 Sept., p. 1202) that such issues are complex and deserving of careful discussion.

Neither my criticisms, nor our symposium, had much to do with young naturalists leading a rebellion against experimentation, or a call for a return to "natural history." Thus, I took exception to a draft of Roush's article that told a story of young naturalists revolting against the approaches of their older, experimentalist mentors. The draft included quotes from esteemed experimentalists—some of whom I had cited as instrumental to the development of experimental ecology—which were clearly at odds with my supposed views. I called Roush to respond to his draft. I told that it inaccurately represented the symposium and our views, and that there was, in fact, no generational controversy about the role of experiments in ecology. I asked that he revise the piece to reflect the issues we had discussed and that he remove an introductory vignette that highlighted a nonexperimentalist's views that were extreme and, hence, did not fairly represent the symposium. Barring this, I insisted that references to the symposium and our quotes be removed from the piece, because the story that he said he was authoring was about broader issues surrounding experimentation in ecological research, not about resurging interest in natural history, a bias retained in the published article.

Further, it is disturbing that Roush ignored many constructive remarks I made in multiple interviews and that he chose to highlight—in a highly contrived, negative paragraph that distorted other statements we had made in an explicitly constructive way—part of a statement I made in an off-the-record conversation (not in one of the three interviews). My comment came at the end of a frustrating, 72-minute conversation (initiated by me) in which I tried to convince an unwavering Roush of the inaccuracy of his draft. I made an unfortunate, blunt statement emphasizing that there are both older, seminal experimentalists who rooted their studies in natural history and many young ecologists who do experiments without the benefit of same, that is, that controversy between young naturalists and old experimentalists was imagined. I then contacted Roush's editor.

After I conveyed these concerns to the editor, the introductory vignette was deleted, and additional emphasis was to have been placed on other issues (experimental design, multiple causality, and so forth) discussed in the symposium. I suggested that a historical synopsis of ecology as a discipline would be a logical replacement introduction, but the editor dismissed this as "not engaging." *Science*'s interest in provoking controversy rather than in telling a factual story about experimental ecologists of all ages and career stages taking a hard look at experimentation in our discipline—while ignoring indications from me and other ecologists that the story was inaccurate—is at best, regrettable. Curiously, the editor refused my repeated requests to review the final version of the article. This is particularly disconcerting in light of assurances to me by Roush and his editor that *Science*'s motivation was to publish an accurate piece and their repeated thanks for my efforts to ensure this goal. Such an article would have

been informative and easy to write, given the diversity of ecologists with whom Roush spoke and our symposium proposal that provided the necessary background. It is unfortunate that the article took such a narrow view both in topic and in highlighting my comments, particularly since it was the lead article in a special issue devoted to ecology.

Joseph Bernardo
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References

1. J. Bernardo, W. J. Resetarits Jr., A. E. Dunham, *Science* **268**, 1065 (1995).

Response: We invited Bernardo and other knowledgeable ecologists to comment on our article and we made changes based on their comments. As Bernardo points out, we even removed a vignette about a researcher with whom Bernardo disagreed. It was not appropriate, however, to shape the entire article to reflect Bernardo's views, which his letter makes clear was his intent.

Bernardo and Resetarits say that we ignored their efforts to focus the article on experimental design. Yet the article highlights their own comments and those of other scientists on some of the very issues such as multiple causality and inference they raise in their letters. And although they object to our portrayal of the roots of the debate, it was supported by other researchers, some of whom were quoted by name in the article. No one told Resetarits that the strides made in ecological experimentation were "not engaging"; indeed, the article included a long section describing those strides.

It is unfortunate that Bernardo now seeks to distance himself from one of his many "blunt statements" by saying it was made off the record. At no point in our discussions, including the interview he initiated, did Bernardo request that we not quote him.

We regret that the idea of researchers seeking value in myriad experimental approaches did not come across more clearly in the article. We agree with Power *et al.* that ecology is a rich and important field and intend to continue our coverage of it. Our intent in this article was certainly not to provoke controversy, as Bernardo asserts. As these letters, and letters we published on 1 September, indicate, ample controversy already exists.

—***Joshua Fischman, Deputy News Editor***

AIDS Intervention in Uganda

Rachel Nowak, in her News article "Testing AIDS interventions: When is the price too high?" (8 Sept., p. 1334), suggests that our study in Rakai District, Uganda, which uses intensive control of sexually transmitted diseases (STDs) through mass treatment as a means of preventing HIV (human immunodeficiency virus) transmission, "runs counter to internationally accepted guidelines." The basis for this statement is that the international guidelines recommend that should the therapy prove efficacious, it should " 'be made reasonably available to the inhabitants of the host community or country,' " and Nowak writes that "If the intervention works, most Africans may not be able to afford the drugs."

Drug costs are a relevant issue, but many of those used in the Rakai study are cheap, readily available in Uganda, and appropriate to the Ugandan context. Two drugs, Azithromycin and Ciprofloxacin were selected for their high rates of effectiveness against key STDs and their ease of administration, and their prices have been falling in the United States. Azithromycin now costs approximately $9.50 per course of treatment, which is comparable to other recommended prescription regi-

mens for chlamydia and chancroid. Ciprofloxacin, a recommended treatment for gonorrhea, costs only $1.75 per course.

The Rakai study is not unique; mass treatment with Azithromycin is also being evaluated in a clinical trial of trachoma prevention in several African countries. Historically, therapies such as Ivermectin for river blindness or hepatitis B vaccine, which were expensive during the research phase, are now affordable and widely used. Thus, research costs cannot necessarily be extrapolated to programmatic costs when potential applications are assessed.

The Rakai study was designed to determine the impact of intensive STD control on HIV incidence and to identify which STDs are most strongly associated with HIV transmission. It is an efficacy trial designed to provide the scientific basis for policy and is not intended as an effectiveness study to test an operational strategy per se. The findings from the Rakai study will be used to devise rational, targeted strategies that can be evaluated by operational research. We believe that sound policy should be based on solid science and that the scientific findings from the Rakai study will be applicable to intervention programs in Uganda and elsewhere.

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■

Additional Reference

We wish to call attention to an additional reference that is relevant to our recent report demonstrating magnetic resonance imaging of elastic properties of materials, on the basis of visualization of acoustic shear waves (29 Sept., p. 1854). Winfried Denk and colleagues have used a method that employs similar motion-sensitizing gradients to observe oscillatory flow of fluid in the cochlea in response to applied longitudinal acoustic waves (*1*).

Richard L. Ehman
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References

1. W. Denk *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 1595 (1993).

Corrections and Clarifications

In the letter "Asymmetrical ability" by G. Hickok *et al.* (13 Oct., p. 217), the two sets of means and standard deviations that appeared in parentheses were inadvertently interchanged. In line 11 of the second paragraph, the mean should have been −0.34 and the standard deviation, 0.14. In line 20 of the same paragraph, the mean should have been −0.23 and the standard deviation, 0.24. Reference 1 should not have been cited in the first sentence of the second paragraph.

Letters to the Editor

Letters may be submitted by e-mail (at science_letters@aaas.org), fax (202-289-7562), or regular mail (*Science*, 1333 H Street, NW, Washington, DC 20005). Letters will not be routinely acknowledged. Full addresses, signatures, and daytime phone numbers should be included. Letters should be brief (300 words or less) and may be edited for reasons of clarity or space. Beginning in October 1995, our previous policy of consulting with all letter authors before publication will be discontinued.

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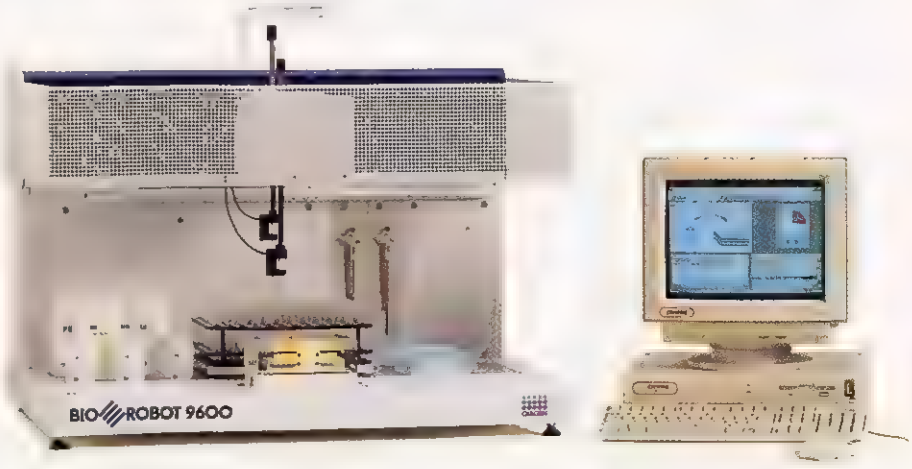

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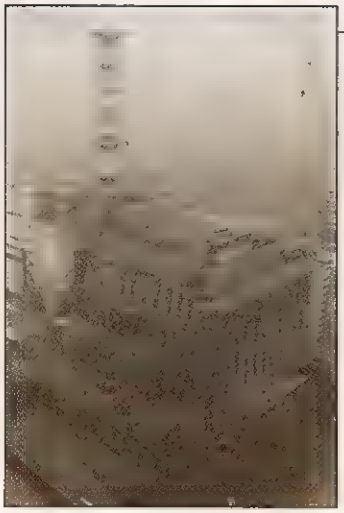

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Doomed? "Sarcophagus" over Chernobyl reactor, where scientists work is on the verge of collapse.

Chernobyl Lab's Destiny Uncertain

A unique research lab inside the destroyed Chernobyl nuclear reactor may have to be abandoned, depending in part on what happens next month at a meeting of Western countries intent on averting another radiation disaster.

The explosion at Chernobyl in April 1986 killed 31 people at the site and spewed radioactive particles now blamed for a reported rise in the incidence of thyroid cancers in children in Belarus. To prevent the escape of more radioactive material, Soviet engineers built a concrete "sarcophagus" over the damaged reactor. Ukrainian scientists set up shop inside to study, among other things, a unique, lethally radioactive mineral formed from molten nuclear fuel.

But the scientists in the sarcophagus now have more than radioactivity to fear. Last July, a consortium of experts hired by the European Commission concluded that the 300,000-ton structure would collapse in a strong earthquake. The collapse would level the lab and spew radioactive dust over a "significant" area around Chernobyl, states a report from the Alliance consortium, led by the French firm Campenon Bernard SGE.

The report recommended that a new concrete shelter be built over the sarcophagus. A high-level nuclear policy panel that advises Ukrainian President Leonid Kuchma backs the plan. A new shelter, says chemist Valery Kukhar, who chairs the panel, would let scientists continue their work in the sarcophagus and could allow Ukraine to clean up the site. But the shelter would cost about $1 billion—a price Ukraine's struggling economy can't afford, Kukhar says. Other scientists have suggested simply filling the sarcophagus with concrete.

Because Western Europe would foot most of the bill, it is expected to decide the fate of the damaged reactor in a meeting of a working group of the G7—an economic alliance of four Western European countries, the United States, Canada, and Japan—in Kiev early in November.

Japan Envisions New Accelerator

Two Japanese institutes are laying plans to boost their country's role in elementary particle physics by building a $700 million, 50-GeV accelerator to produce K mesons, or kaons.

The new facility will be based at KEK, the Institute for High-Energy Physics, in Tsukuba, and run jointly with the University of Tokyo's Institute for Nuclear Science. Columbia University's Shoji Nagamiya, who aired the plan this month at a physics meeting in Santa Fe, New Mexico, says it will be used to study high-energy kaons, pions, and heavy ions. An added feature—and one that raised the cost—is the capability to shoot a stream of neutrinos into a cavern 250 kilometers away at the soon-to-be-completed SuperKamiokande neutrino detector, an experiment expected to help determine whether neutrinos have mass and if so, how much.

Peter D. Barnes, physics director at Los Alamos National Laboratory, says such a facility "has been talked about for a number of years, but other countries were not able to make it happen." Proponents hope to win initial funding in Japan's 1997 budget, which would permit completion of the project by 2002. Plans call for the construction of the accelerator to be a domestic project, but international scientific involvement will be on the agenda at a December workshop in Tokyo.

Britain to Keep Science Committee

The British government's surprise decision this summer to reorganize its top science office is still provoking controversy, as a debate in Parliament last week showed. One question the shakeup had left hanging—whether to abolish Parliament's science and technology committee—now appears to have been settled, with the committee's future safe.

The science and technology committee was set up following the creation of the Office of Science and Technology in 1992 to offer the government advice on science policy. Among its works are a report on human genetics, completed earlier this year, and a review of Britain's research councils, to begin this fall. But its future was in doubt following the sudden shift of the OST from the Cabinet Office to the Department of Trade and Industry (DTI) in July.

Last week, in the first parliamentary debate on science since the shift, many members questioned the wisdom of the reorganization, which critics fear will tie science too closely to industry. But they could at least take comfort that Ian Lang, president of the Board of Trade and the Cabinet minister now responsible for science, said that the government wished to continue the independent parliamentary panel, which Lang said "has tackled a variety of important topics and played a considerable role in drawing [attention to] scientific and technological issues."

John Mulvey, spokesperson for the lobbying group Save British Science, welcomes the decision to keep the committee but stresses the scientific community's continuing worries about the move of OST to DTI. Noting that DTI is to reply soon to the genetics report, which focuses on medical and ethical—not commercial—issues, he says, "It's absurd that the president of the Board of Trade should respond."

Budget Chief Sees Steady State for Basic Research

Basic research should emerge relatively intact from the current budget battle, says Alice Rivlin, director of the Office of Management and Budget, and it has a high priority in next year's presidential budget request.

"We're trying to hold the line [in 1997]," Rivlin told members of the President's Committee of Advisers on Science and Technology (PCAST) this week in a preview of what the Administration is planning for the fiscal year that begins on 1 October 1996. "While other agencies are looking at cuts of 20% or more, we're hoping to maintain current levels [for basic research]. That's the best we can do."

PCAST members wanted more, however, especially for the National Science Foundation (NSF). "I was hoping that she would see the important role that NSF plays in funding basic research as a reason for increasing its budget," said panel member Philip Sharp of the Massachusetts Institute of Technology. Panel members also urged Rivlin to boost energy R&D to reduce U.S. dependence on foreign oil.

As for this year's budget wars, Rivlin predicted that the White House and Congress will reach agreement "sometime between Thanksgiving and Christmas" on individual 1996 spending bills. The key, she said, is moving some or all of the $7 billion that Congress has added to defense into high-priority social programs, a compromise that would allow both sides to keep their promise to hold down government spending.

Max Planck Institutes Brace for Change

With funding for life and no teaching requirements, top Max Planck researchers are in an enviable position. But tight budgets and a new generation may bring reforms in the system that some say are overdue

MUNICH—For the Max Planck Society (MPS), the announcements earlier this month that two of its researchers had been awarded Nobel Prizes was cause for celebration. But it probably wasn't a great surprise. In fact, Germany's premier basic research organization is getting used to sharing in the October honors: This year's awards to developmental biologist Christiane Nüsslein-Volhard and atmospheric chemist Paul Crutzen (*Science*, 20 October, p. 380) bring to 30 the number of Nobelists who have worked at Max Planck Institutes (MPIs). Ten MPS researchers have won the prize in the past 11 years alone.

Few research organizations can boast such a record. But then, few provide the kind of unfettered environment for doing science that the MPS offers. Its senior scientists, known as directors, get lavish support for life—freeing them from the pressures of grant applications, university committees, and teaching. Indeed, the MPS has been so successful that several organizations, including the Howard Hughes Medical Institute in the United States and Britain's Wellcome Trust, have adopted its idea of strongly supporting a few outstanding researchers.

With this track record, any talk of changing the MPS might seem misguided. Yet some scientists say change is needed—especially given the country's new circumstances. As the MPS expands into former East Germany and helps build up its science, research money in the west is much tighter. That makes it harder to keep—and to justify—a system where directors get funds for life, regardless of their productivity. Reformers also favor doing more for young scientists and relaxing the strong focus on directors. "The Max Planck is very old-fashioned in the way [directors] are selected, get money, the way quality is controlled," says Nüsslein-Volhard, a director at the MPI for Developmental Biology in Tübingen. "They treat you like you're a genius, whether it's true or not."

All these pressures are mounting just as MPS's top echelon is about to turn over. A wave of retirements will see one quarter of the 222 directors replaced by a new generation over the next 5 years. And zoologist Hubert Markl, the first "outsider" ever elected MPS president, will take over next June (see box). That's why, says one MPS scientist, "it's now or never if we want to change the system."

Eastern expansion

Created in 1911 as the tiny, elite Kaiser Wilhelm Society, the MPS has over the years built institutes around some of the century's leading scientists, including Albert Einstein, Otto Meyerhof, and Werner Heisenberg. Today it is big business: MPS supports 69 institutes with a 1995 budget of $1.2 billion from the federal and state governments. That puts it on a par with Germany's largest granting agency. And there's the dilemma, says Ernst-Ludwig Winnacker of the University of Munich (and associate member of the MPI for Biochemistry in Martinsried)—"whether the current [MPS] structure, devised in 1911 to build institutes around a few genius scientists, can always be applied today."

MAX PLANCK SOCIETY'S NOBELISTS

Max von Laue	1914	Physics
Richard Willstätter	1915	Chemistry
Fritz Haber	1918	Chemistry
Max Planck	1918	Physics
Albert Einstein	1921	Physics
Otto Meyerhof	1922	Medicine
James Franck	1925	Physics
Otto Warburg	1931	Medicine
Carl Bosch	1931	Chemistry
Werner Heisenberg	1932	Physics
Hans Spemann	1935	Medicine
Petrus Debye	1936	Chemistry
Richard Kuhn	1938	Chemistry
Adolf Butenandt	1939	Chemistry
Otto Hahn	1944	Chemistry
Walther Bothe	1954	Physics
Karl Ziegler	1963	Chemistry
Feodor Lynen	1964	Medicine
Manfred Eigen	1967	Chemistry
Konrad Lorenz	1973	Medicine
Georges Köhler	1984	Medicine
Klaus von Klitzing	1985	Physics
Ernst Ruska	1986	Physics
Robert Huber	1988	Chemistry
Johann Deisenhofer	1988	Chemistry
Hartmut Michel	1988	Chemistry
Erwin Neher	1991	Medicine
Bert Sakmann	1991	Medicine
Christiane Nüsslein-Volhard	1995	Medicine
Paul Crutzen	1995	Chemistry

That question had already been in the air "for ages," says one former MPS researcher, when the fall of the Berlin Wall confronted the MPS with new realities. After East and West Germany were reunited in 1990, the government looked to its research organizations to help rejuvenate the east's dilapidated science and pressured the MPS to open new institutes quickly. But the MPS leadership balked at abandoning the society's policy of starting institutes only where strong science and a good local infrastructure already exist, says the MPS's current president, law specialist Hans Zacher. The result was a compromise: First, the MPS would set up small research groups around promising university scientists for 5 years. Then, as local conditions improved, they would build institutes—all with new money, saving MPIs in the west from massive cuts.

Initially, universities and scientists welcomed the 27 new groups. "Compared to the [East Germany] days, this is almost like a dream," says Johann Dorschner of the MPS astronomy group at the University of Jena. But the honeymoon didn't last long. With the first groups nearing the end of their 5 years' support, the universities have backed away from promises to absorb group members and not just the leaders, says Zacher. And that threatens to break up teams just as they are hitting their stride. With no overall solution in sight, the MPS and universities are negotiating person by person, says Zacher, and the MPS will support a third of the remaining people for three more years.

Meanwhile, the MPS has founded nine institutes in the east, in fields from gravitational physics to the history of science. Plans for two more are approved, and the goal is to establish another five or six, says Zacher. But it has been tough going. "It's difficult to attract [outside] people to the former east," says neuropsychologist Angela Friederici, who left a professorship at Berlin's Free University for an MPI directorship in Leipzig. "It will take time for the universities to reach the level of those in western Germany," she says.

Retrenchment in the west

Back west, reunification has meant tighter money for public institutions across the board, and the MPS is no ex-

Hubert Markl: Animal Behaviorist Puts His Learning to Work

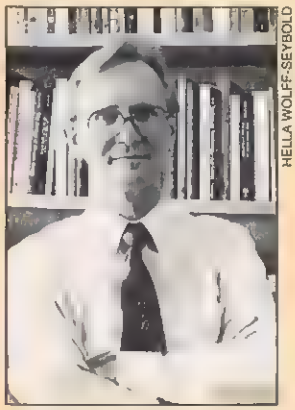
BERLIN—At a time when the pressure for change is buffeting the Max Planck Society (MPS) from many sides (see main text), Germany's foremost research organization has for the first time chosen someone from outside its own ranks to take the helm: zoologist Hubert Markl. He may be an "outsider" to the MPS, but Markl is no stranger to the German scientific community. While holding down a professorship at the University of Konstanz near the Swiss border for the past 2 decades, Markl has also been a newspaper columnist, an essayist on scientific ethics, the head of Germany's main granting agency, and president of the reorganized Berlin-Brandenburg Academy of Science—the successor to the prestigious former Prussian Academy of Science.

Colleagues describe him as a formidable intellect, a "spellbinding speaker," a philosopher of science, a good administrator, and "a politician in the best sense of the word." He will need all those talents when he takes over the MPS presidency next June, as the organization struggles to cope with tight budgets and the challenge of opening new institutes in Germany's eastern states.

Born in Bavaria in 1938, Markl studied science at the University of Munich, earning his Ph.D. in zoology in 1962. He did postgraduate work at Harvard University and Rockefeller University in 1965–66, and directed a German zoological institute before becoming a biology professor at Konstanz University in 1974. Much of his research has focused on animal communication. He has also studied how some insects develop complex social systems from simple beginnings, and is fascinated by the way individual ants and bees contribute to their complex hives and colonies by "optimizing their behavior and their goals." It is an observation he keeps in mind when analyzing human organizations. As a leader, Markl said in an interview with *Science*, he tries to ascertain "whether something you want to achieve is better achieved if you just let the individuals do their thing, or whether you have to impose centralized planning. ... Leadership from the top, in conjunction with 'bottom-up' independence, can provide the best solution."

Markl has served on the governing board of the DFG, Germany's basic research granting agency, since the 1970s, and was the organization's president from 1986 to 1991. In 1993, as the Berlin authorities struggled to weld together the scientific traditions of east and west, Markl became founding president of the Berlin-Brandenburg Academy, a post he relinquished last month. There he helped organize interdisciplinary working groups that joined prominent scientists from both sides of the old border. "The greatest challenge was to bring together scientists with such different biographical backgrounds into a situation where they can work together again," Markl said. Detlev Ganten, who also had to grapple with merging east and west as head of the Max Delbrück national research center near Berlin, says Markl "mastered the situation." Ganten describes Markl as "politically savvy, yet able to project freshness and openness."

Those skills attracted the attention of a search committee of Max Planck's governing board, whose 55 members cast written ballots this summer to ratify Markl as the new president. While Markl is hesitant to define specific plans for the MPS before his term as president begins, he told *Science* in two recent interviews that he wants "to make sure there will be more emphasis on concentrating resources in centers of excellence" and more clearly defining the missions of scientific institutions. He also wants to foster more cooperation and joint projects between Max Planck institutes and the traditionally separate university system. And he advocates a bit more freedom for talented young scientists chafing to do independent research, although he says such decisions should be made on a case-by-case basis.



HELLA WOLFF-SEYBOLD

Insiders' outsider. Markl, the first president from outside the MPS, will take up the chain of office *(left)* in June.

Markl makes clear that he plans to help encourage more women to rise as scientific researchers. "We have to consider this as a major challenge in the next decade," said Markl, who sees far too few women in the higher levels of the MPS. "Things are moving, but they are moving glacially."

As for the east, Markl wants the MPS to establish enough new institutes so that—by the turn of the century—the representation in the east will be roughly proportional to that of western Germany. But he concedes that, if German federal and state governments do not live up to budget commitments, then "it will be very difficult" to bring eastern Germany to that level.

"Max Planck was founded to be ... as good as any institution in the world," Markl says. "To do the best research that can be done, to attract the best people, and give them the best opportunities. This will be my major goal."

–Robert Koenig

Robert Koenig is a science writer in Berlin.

ception. For an organization used to "swimming in money," says an MPS astronomer, the change has been tough. So far, savings have been made by closing selected research areas as their directors retire, says Zacher, a trend that will continue. Beyond this, all institutes face staff cutbacks—a worrisome solution, says Steven Beckwith, a director at the MPI for Astronomy in Heidelberg, as it hits mostly young scientists on fixed-term contracts. "If we cut back positions, my whole group vanishes," he says.

But some researchers believe that the system could benefit from a bit of belt-tightening. Molecular biologist Benno Müller-Hill of the University of Cologne carried out a detailed comparison of two MPIs with 11 other German and foreign research departments and institutes and found that the MPS system costs more than twice as much to produce highly cited papers as, for example, his own university department, the Cold Spring Harbor Laboratory on Long Island, New York, or Heidelberg's European Molecular Biology Laboratory.

One consequence of the squeeze is that future cuts will be tied more closely to research productivity, says Thomas Trautner of the MPI for Molecular Genetics in Berlin, one of four MPS vice presidents. "There is a broad consensus among Max Planck directors that this is the way to proceed," he says.

Along with the debate on cutbacks, another long-standing taboo subject is being openly discussed: the idea that directors should receive part of their funding through quality assessment or project proposals. Although no sudden policy shift is likely, says one insider, "the discussion is heating up."

Says one MP biologist who requested anonymity: "You can't go on funding research with little coming out at the end."

On the other side, supporters of the status quo say it promotes harmony. "At the moment, the pie is divided rather equitably," says Ken Holmes, a director at the MPI for Medical Research in Heidelberg. "I prefer this to the kind of feuding that would come with [a more formal review system]. It won't save enough money to make it worthwhile." Others argue that freedom from competition for funds allows directors to start risky projects without being under pressure to publish.

Small is beautiful

Perhaps the thorniest issue for the MPS is the tradition of powerful directors building up research groups that can reach the size of a typical university department. Several physicists interviewed by *Science* argued that big groups are crucial for some large-scale projects. But many biologists felt that institutes with lots of independent groups using different systems are more in tune with the times. "People want independent colleagues, not lots of junior groups dependent on you," says Nüsslein-Volhard.

And at present there are few chances for young people to be formally independent. "[The MPS] can be very stifling to up-and-coming scientists," says biochemist Walter Hill of the University of Montana, an adviser to Berlin's MPI for Molecular Genetics. "It throws a blanket over other people ... [and] encourages them to become puppets of the director." Of the MPS's 2800 scientists below director level, only 30 are officially independent group leaders with their own resources.

Working under a director has been fine for some. Take Hartmut Michel, now a director at the MPI for Biophysics in Frankfurt. Michel was working in Dieter Oesterhelt's group in the early 1980s when he took on a project other scientists thought was impossible: crystallizing a protein from the cell membrane. His work on the photosynthesis reaction center won him and two MPS collaborators the 1988 Nobel in chemistry. Michel says that continuous funding and topnotch facilities were "absolutely decisive" to his success. "[The MPS] makes your life as easy as possible," he says. Similarly, neither Bert Sakmann nor Erwin Neher were formally independent when they developed a technique for measuring the flow of ions through single nerve channels—research that earned them the 1991 Nobel Prize in medicine.

But for the less lucky ones, life at an MPI can be frustrating or—in extreme cases—disruptive to their careers. When a director retires or dies, says one MPS researcher who requested anonymity, "the leftover people all scramble for a foothold." And, although there are many such "leftover" staff—some 20%, according to several estimates—they have no real place in the system. In fact, some say the institutes often try to push them out, regardless of the quality of their work. "I don't expect a free lunch," says one researcher. "What I object to is getting kicked in the butt."

Some MPS leaders dismiss criticism of these harsh realities. "The director has a lot of freedom to structure his department," says Vice President Trautner. "This is entirely adequate to encourage young people." To avoid leftovers, he favors drastically reducing the number of permanent positions for non-directors. Others see a solution in creating a tenure-track, middle level of independent researchers. Besides nurturing young talent, says Nüsslein-Volhard, it would allow institutes to cover more areas. And it would prepare more women for top posts in the MPS, she says, where they are abysmally underrepresented—a problem common throughout German research.

With change in the air, it is perhaps the perfect moment for a man like Markl to take over: someone not steeped in MPS traditions, yet a formidable intellect and a skillful politician. What's more, says Nüsslein-Volhard, "he might not assume we're all geniuses."

–Patricia Kahn

With reporting from Robert Koenig.

PLANETARY SCIENCE

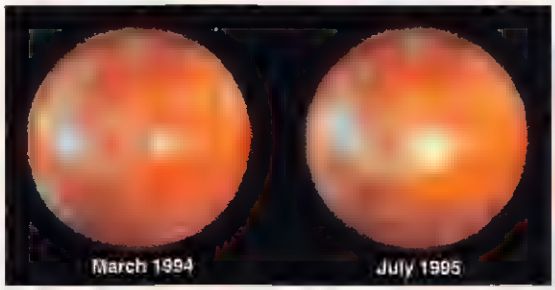
Galileo Lives With Balky Tape Recorder

Sooner or later, it happens to everyone who has a tape recorder. You push the button on your machine and nothing happens. A moment later, you try again, and it works. Engineers operating the Galileo spacecraft that is nearing Jupiter know the feeling. On 11 October, they found that the recorder that is supposed to store data for eventual transmission to Earth was stuck. Just like frustrated audiophiles, they backed off and tried their tape recorder again. Now it seems to be working—to the engineers' great relief.

"We have a good idea of what the problem is and good ideas of how to work around it" to keep the tape recorder rolling smoothly, says manager William O'Neil of the Jet Propulsion Laboratory (JPL) in Pasadena, California. If he's right, Galileo will once again have dodged a technical obstacle threatening the $1.3 billion mission.

The balky tape recorder is central to plans for making up for the failure of Galileo's main communications antenna to open fully (*Science*, 5 February 1993, p. 759). Using the latest data compression techniques to compensate for the reduced transmission rate, Galileo will be able to return about 70% of the data that mission planners had hoped for. But that will only be possible if some of the big data loads can be stored for long enough to allow the crippled communications system to catch up.

By late last week, engineers had concluded that the tape recorder probably malfunctioned because its moving parts had stiffened up, says O'Neil: "One of the problems could be that we have used this tape recorder so infrequently." Besides a sticky mechanism, the machine also suffered from the mechanical disadvantage of a nearly empty reel pulling on a full one when controllers sent the order to rewind. Last Friday, when engineers commanded it simply to play back—the easy direction to drive the tape—it worked fine. So engineers plan to limber up the mechanism by working it through a series of exercises; to be safe, they will also avoid recording or playing near the end of the tape, says O'Neil.



Out of sight? Without a tape recorder, Galileo could not image this new volcanic spot *(right)* on Jupiter's moon Io.

If that regimen doesn't work, says project scientist Torrence Johnson of JPL, the mission would lose another 20% of the data it was originally expected to gather, with imaging of Jupiter and its satellites suffering the brunt of the losses. The losses would be even greater, says Johnson, if not for the new data compression techniques and a contingency plan to use the on-board computer for data storage.

Still, engineers would be resting easier if Galileo carried a spare tape recorder. But Johnson explains that when the Galileo mission was being planned, its managers "couldn't sell the necessary extra mass and expense. ... Every bit of incremental growth in Galileo was regarded as a dire threat to its existence." And that leaves the Galileo team with nothing to fall back on but innovation. Claims O'Neil: "We've demonstrated we're the most resilient planetary mission ever flown."

–Richard A. Kerr

EUROPEAN SPACE AGENCY

All Aboard the Space Station

TOULOUSE, FRANCE—Until almost the last minute, Europe's involvement in the international space station had hung in the balance, threatened by conflicting priorities and ailing economies in the member nations of the European Space Agency (ESA) (*Science*, 13 October, p. 224). Last week, however, the suspense finally ended when a 3-day meeting of government ministers in this southern French city managed to cobble together a set of compromises that will allow Europe to hop aboard the space station, joining the United States, Canada, Japan, and Russia.

In a surprising accord, nine of ESA's 14 member countries agreed to contribute a total of $3.5 billion to the station between now and 2004. Even Italy, whose budget crisis had posed the most recent threat to Europe's space station ambitions, will be aboard, thanks to a series of unorthodox financial measures including a proposed commercial bank loan. The Toulouse meeting also saw a series of compromises on other disputes threatening ESA's unity. But amid the wheeling and dealing, there was one clear loser: ESA's thriving program of scientific space missions.

For most participants, that didn't dampen the exultation. "If we had not succeeded, ESA would have disappeared the following morning," French technology minister François Fillon told a gathering of journalists during the meeting. "I think we all won," agreed Jürgen Rüttgers, Germany's minister for education, science, research, and technology. All but ESA Science Director Roger Bonnet, that is, who is facing the loss of up to 15% of his program's budget over the next 5 years. Reportedly angry at the outcome, Bonnet refused to speak to journalists after the meeting.

Much of the groundwork for the agreement in Toulouse was laid in a series of meetings between Rüttgers and Fillon over the past few months. France, whose interest in the space station has always fallen far short of Germany's keen enthusiasm, agreed to boost its participation to 27.6%, a major increase over the roughly 10% it had been offering until quite recently. As a quid pro quo, Germany agreed to more than double its subsidy for a separate part of the ESA program, the new Ariane-5 rocket, whose construction is an economic boon to France.

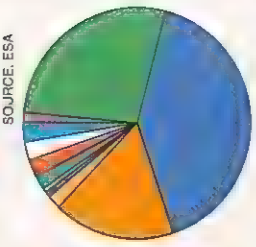
It was not until the Toulouse meeting, however, that ESA ministers came up with a solution to the most immediate threat to their station plans: Italy's insolvency. Italy had thrown a monkey wrench into the works at the end of September when it announced that it lacked more than a third of the $350 million ESA expected it to contribute to space station development through 2000. In a plan worked out in Toulouse, which must be approved by the Italian government, ESA would cover half of this shortfall by making economies in the space station program, while Italy would take out a bank loan to cover the other half. To sweeten the pot for Italy, France and Germany promised to cede it almost $80 million in industrial contracts for the station.

In the scramble to gather enough funds for the space station, however, ESA ministers approved a German proposal to cap the science budget at its current level, about $460 million per year, for the next 5 years—and this sum will only be adjusted for any inflation above 3% a year. As inflation in the ESA countries is currently running at least this high, the science program may well see its purchasing power decline by as much as 15%.

The news could have been worse: The ministers did reject a British demand for a 25% cut in the science budget over the next 5 years. And officials gave contradictory assessments of the likely effects of the budget freeze. ESA's director-general, Jean-Marie Luton, told journalists that a series of economy measures—notably a 12% cut in the agency's overall administrative costs over the next 3 years—means that "if all goes well there will be no delays in scientific missions." Yet Ian Corbett, science director of the U.K. Particle Physics and Astronomy Research Council, told *Science* it would be "accurate" to regard the ministerial decision on the science budget as an actual cut in funds, which could put a squeeze on planned missions. On the other hand, said Corbett, "we believe that Mr. Bonnet will be able to deliver the program he's promised."

The ministers did agree to review the consequences of the science freeze at their next meeting in Brussels, scheduled for late 1997 or early 1998. But any modification would have to be approved by all 14 member countries. In the meantime, Bonnet's silence about the fate of his program may be speaking louder than words.

–Michael Balter



Total = $3.53 billion

All for one. European contributions to the space station from 1996 to 2004.

POLITICS

Clinton Defends R&D in Partisan Speech

Support for science used to be something that both Democrats and Republicans could agree on. Not anymore. Since last fall's congressional election, science has become a bitterly partisan issue, with each party accusing the other of backing the wrong sorts of programs in a search for sustained economic prosperity.

The latest evidence of that animosity was a speech last week by President Bill Clinton, on the occasion of the awarding of the National Medals of Science and Technology (*Science*, 6 October, p. 35). The president denounced what he labeled "drastic cuts" by Republicans in the $72 billion federal R&D budget, which is divided among several 1996 appropriations bills now languishing in Congress.

AP/W DE WORLD PHOTO

Tough talk. Clinton greets Ohio senators Mike DeWine and John Glenn after awarding medals.

"The plan now being considered by the Congress will cut vital research and development by a third," said Clinton in his first speech as president devoted entirely to science. "We could have a balanced budget to show for it tomorrow, but a decade or a generation from now our nation will be much the poorer for doing that." Vice President Al Gore, who introduced the president, took a sharper tone, calling Republican cuts to science and technology "unwarranted, unwise, and unnecessary" and warning that the entire U.S. R&D enterprise is under attack.

That analysis did not sit well with Republican leaders, who did not attend the White House event. "The president is defending the way things have always been, and he has no designs to accommodate science programs to the changing world," said Representative Robert Walker (R–PA), chair of the House Science Committee. "We're looking 20 to 25 years in the future," Walker told

Science. "He's looking backward."

Democrats in the House and Senate welcomed the speech, but some want Clinton to take an even stronger stand by saying that R&D is a high priority in deciding whether to veto a specific bill. Representative George Brown (D–CA), the ranking minority member of the House Science Committee, said that he and other minority lawmakers planned to meet this week with Clinton Chief of Staff Leon Panetta to make their case. But Brown was annoyed with the fact that they must first have an audience with Panetta before meeting with the president. "That in itself shows we don't have a hell of a high priority," Brown says.

The mere prospect of a presidential speech on science and technology set off a scramble among agencies and White House officials. Commerce Department officials, for example, sent their own version of the talk to Clinton's speechwriters; they wanted the president to be more critical of cuts in specific programs, according to Administration sources. Meanwhile, staffers at the Office of Science and Technology Policy—who wrote the first draft of Clinton's message—favored a speech that emphasized broad themes without getting into nitty-gritty budget details. The result appeared to be a compromise between the two approaches, with Clinton mentioning Commerce's $430 million Advanced Technology Program that is threatened by Republicans, while also speaking generally about the importance of science and technology.

At the same time, White House officials defended their boss against criticism that R&D programs are not important in making veto decisions. "Our commitment on this issue has been clear from day one: We are philosophically opposed to people cutting out whole areas of research," says Greg Simon, Gore's domestic-policy adviser. He said that science and technology issues are discussed daily in meetings with senior White House officials and that the Administration has made a major effort to preserve the Commerce Department (*Science*, 22 September, p. 1664). "I don't know what else to do," he quipped, "[except] have a march on Washington." Senator John Glenn (D–OH) also defended the president, saying that he's convinced the White House will fight for R&D programs at the bargaining table.

With congressional supporters of R&D clamoring for attention, the president's aides emphasized that the fate of research programs is only part of a broader battle between the White House and Republicans. "The whole house is on fire," says Simon, "and [science and technology] is just one room."

–Andrew Lawler

NEUROSCIENCE

Center for the Mind Pleases the Senses

LA JOLLA, CALIFORNIA—Picture this: a scientific institute with 32 relatively young fellows, all of whom receive ample funds and have no need to apply for grants. No one has any teaching responsibilities. Theoreticians and experimentalists alike are welcome and nurtured. Lunch is served each day in a communal dining room, where the fellows can mix with an endless stream of distinguished visiting researchers. Now set all of this in a $16 million architectural wonder discreetly built into a hillside on the Torrey Pines Mesa that is already home to the Scripps Research Institute, the University of California, San Diego, and the Salk Institute for Biological Studies. Sounds like a dream? Well, it was until 15 October, when the Neurosciences Institute (NSI) cut the ribbon on what it bills as its new "monastery for science."

The abbot is Gerald Edelman, who shifted into neuroscience after winning a Nobel Prize in 1972 for his work in immunology. Edelman's monastery has been a long time evolving. It began in 1981 as an offshoot of the Neurosciences Research Program at the Massachusetts Institute of Technology. Edelman, then at Rockefeller University in New York, was appointed head of the nascent NSI, a retreat where scientists could discuss a specific idea with a few colleagues for a few days. "It's been a way to get away from quotidian projects, like answering the phone," says biochemist W. Einar Gall, research director of NSI since it started. "It's a very nice opportunity to get together in a workshop setting," says Carla Shatz, a Howard Hughes Medical Institute investigator at the University of California, Berkeley. "They provide a unique service."

To date, more than 900 scientists from 24 countries have passed through NSI, but the institute is now more than just a retreat for visiting scientists. Beginning in 1988, it began to hire a handful of full-time fellows. Four years later, with Rockefeller in some turmoil as then-President David Baltimore tried to overhaul its structure, Edelman left and took a job as chair of the department of neurobiology at the Scripps Research Institute, which became temporary home while NSI laid plans for a more grandiose center across the street. Since then, it has grown to 32 full-time researchers who are fully funded by the institute to pursue their individual projects for up to 4 years. Chosen for their scholarship, creativity, and problem-solving skills, the fellows have a range of interests that, NSI boasts, "represent practically every field of modern neuroscience."

BILL TIMMERMAN

Brainy design. NSI's eye-catching new buildings opened last week on a mesa outside La Jolla.

NSI is largely funded by the Neurosciences Research Foundation, a nonprofit established in 1962 that is supported by corporations, other foundations, and private donations. Although NSI is an independent institute, Scripps owns its facilities (NSI has a 35-year lease) and footed most of the construction bill. Sandoz Pharmaceuticals also chipped in $5 million for construction costs and promised another $5 million a year for operating expenses, in return for the rights to develop products stemming from NSI research.

Edelman's own work focuses on what he calls "neural Darwinism," the theory that populations of neurons develop individual networks through a Darwinian selection process. But Edelman—who emphasizes that his work is supported by the National Institutes of Health, not NSI—is quick to say that the institute doesn't exist to further his own scientific ideas. "There are guys here who actually believe in neural coding," Edelman says, referring to the theory that neurons are genetically coded to make specific connections, just as transistors are wired in a preset pattern. "I think that's insane. But they're good guys."

The model for the current incarnation of NSI is the Rockefeller Institute—before it became Rockefeller University and lost a culture that makes Edelman wax romantic: "There was a sense that you had a lot of time. The whole style was terribly impressive to me." The Rockefeller of yesteryear, he says, also mixed the old and young. "You could sit next to René Dubos or [Oswald] Avery at lunch. ... That exposure was tremendously influential." On top of these luxuries, Edelman says people weren't pigeonholed. "You were not characterized as a specialist," he says.

Whether the new NSI can create the type of environment Edelman remembers—and whether it can survive without federal funding—is anyone's guess. But at least this dream is going to get a reality test.

–Jon Cohen

HEALTH POLICY

New Studies Trace the Impact Of Tobacco Advertising

A packed press conference held last week showcased two new studies guaranteed to get the tobacco industry's goat. One indicates that cigarette advertising incites adolescents to start smoking—rather than persuading adults who already smoke to switch brands as the industry claims. The second shows that the industry's marketing efforts at least double the risk that certain adolescents would start smoking. Both studies were described at the press briefing by behavioral epidemiologist John Pierce of the University of California, San Diego, who directed the research.

The studies come just as the Food and Drug Administration is spearheading a drive to reduce smoking among children by 50% in the next 7 years. Among the agency's proposed strategies: restrict tobacco advertising. So not surprisingly, the tobacco industry came out swinging. "For people who are truly scientifically orientated, Pierce's study should be an affront to objectivity," says Thomas Lauria, spokesperson for the Tobacco Institute, the industry's lobbying group. "We'll be taking a much closer look at the study because of [Pierce's] blatant advocacy [of smoking restrictions]."

But Pierce told the press conference that his methodology will withstand efforts to discredit the studies. *Science* decided to put his claim to the test by sending the studies to a handful of experts to critique. The verdict was mixed. Some said that the studies—which are notoriously hard to do—failed to distinguish cause and effect. But most were supportive: The first report is "strongly suggestive" that advertising causes teenagers to smoke, "but not conclusive," says epidemiologist Malcolm Maclure of the Harvard School of Public Health. Nevertheless, he adds, it's "certainly enough to justify action."

Persuasive. Promotions like this increased smoking in target groups.

The first Pierce study, due to be published in the November issue of *Health Psychology*, found that since the 1880s each of four major advertising drives correlated with increases in smoking among 14-to-17-year-olds, but only of the sex targeted by the advertising. For example, increases in smoking rates among adolescent women—but not teenage boys—coincided with marketing campaigns for Chesterfield and Lucky Strike in the mid-1920s, which included the famous "Reach for a Lucky Instead of a Sweet" advertisement. The only other times the study found that smoking increased without a major promotion was during the two world wars when soldiers were given free cigarettes.

That study focused solely on advertising. The second, published in the 18 October

Researchers Protest Attack on Tobacco Study

Public-health researchers are protesting what they view as an "unprecedented" case of meddling by Congress in a peer-reviewed research project. Twenty-nine health leaders and academics—including Patricia Buffler, dean of public health at the University of California (UC), Berkeley—signed a newspaper ad last week blasting the House appropriations committee for trying to cancel a National Cancer Institute (NCI)–funded study of pro-tobacco lobbying. The ad attacks by name Representative John Porter (R–IL)—prime mover in the House's vote to boost biomedical research this year and chair of the key appropriations subcommittee—because he inserted language into a report asking that the study be ended.

The study that drew Porter's ire is being conducted by Stanton Glantz, professor of medicine and expert on heart function at UC San Francisco. Glantz has become a thorn in the side of the tobacco industry. In 1994, he testified as an expert opposing industry witnesses in regulatory hearings on the risks of ambient cigarette smoke. This year, he created an Internet file at which anyone can view thousands of pages of memos from the Brown & Williamson Tobacco Company—internal documents that were dumped anonymously in Glantz's mailbox. (To view the file, see **http://www.library.ucsf.edu/tobacco/**.)

M. CONSTANTINI/*SAN FRANCISCO EXAMINER*

Congressional target. Stanton Glantz of UCSF.

In 1993, Glantz won a 3-year, $600,000 grant from NCI to "determine the extent and nature of tobacco industry influence on state tobacco policy-making," according to an abstract. Glantz and his research team have been collecting data on contributions to state legislators by tobacco companies and analyzing the impact on state efforts to control smoking.

Porter inserted language into a report on the National Institutes of Health (NIH) in August, saying that the appropriations committee was "disturbed to learn about" Glantz's study. Porter's press aide, David Kohn, says the congressman learned about it from a newspaper reporter, who appears to have learned of it from pro-smoking lobby organizations. The House report says that "such research projects do not properly fall within the boundaries of the NCI portfolio, especially when nearly three quarters of approved research projects go unfunded." The report calls for the grant to be stopped in this, its second year.

Instructions contained in congressional reports do not have the force of law but are usually obeyed, especially when they come from committee chairs, and particularly if both the Senate and House agree. In this case, the Senate has not endorsed the attack on Glantz's project, but could do so in the final NIH bill, which is still pending in Congress.

Last week's newspaper ad said Porter's move would let "tobacco companies pollute and limit scientific inquiry." But Kohn says Porter regards such criticism as "irresponsible" and "absurd." Kohn points out that Porter—"an 18-year champion of biomedical research"—has voted in the past against tobacco subsidies. He wants NCI to drop this study because it doesn't qualify as genuine clinical or behavioral research, Kohn says.

Kohn adds that Porter would be delighted if NIH could find a way to pay for the study from "other sources," such as a discretionary account controlled by NIH Director Harold Varmus. Indeed, Porter met on 14 September with Varmus and NCI chief Richard Klausner, in an attempt to end the furor amicably. At the moment, neither NIH nor NCI is saying what will happen to the hot project.

–Eliot Marshall

issue of the *Journal of the National Cancer Institute* (*JNCI*), also investigates the influence of peer pressure in inducing adolescents to take up smoking. It is based on telephone interviews with 3536 Californian adolescents who said they had never smoked.

To assess the adolescents' receptiveness to advertising, the Pierce team rated them on a five-point scale according to the answers they gave to a series of questions about, for example, whether they owned or would like to own any cigarette-related promotional items, and what was their favorite cigarette ad. The researchers gauged the influence of peer pressure by asking the adolescents about use of tobacco among their family members and best friends. Finally, adolescents were considered susceptible to taking up smoking if they failed to state unequivocally that they would not in the future try a cigarette in response to two questions—one of which asked "If one of your best friends were to offer you a cigarette, would you smoke it?"

Pierce and his colleagues found that adolescents who were exposed to family members and peers who smoked were almost twice as likely as others to be susceptible to taking up smoking. But even when peer pressure was taken into account, adolescents rated as receptive to advertising were two to four times more likely to be in the susceptible group than those rated as unreceptive. Because a third, as yet unpublished study by the Pierce team indicates that susceptibility predicts which adolescents eventually smoke, the *JNCI* paper concludes "that tobacco marketing may be a stronger current influence ... than exposure to peer or family smokers" in encouraging adolescents to begin smoking.

Maclure says that the study "shows fairly clearly that even when you control for peer pressure, receptiveness to advertising is a major factor" in inducing teenagers to smoke. But both Maclure and epidemiologist Charles Poole of Boston University, another ardent supporter of increased control of cigarettes, disagree with Pierce's claim that the results show that "tobacco marketing was twice as powerful" as peer pressure. Poole also points out that both published studies suffer from "the classic chicken-and-egg problem," making it difficult to separate out cause and effect. For example, he says, in the second study, "kids who are contemplating taking up smoking may be more aware of advertising."

Pierce, however, is unfazed by the studies' limitations. In public health, he says, "if something appears dangerous we pull it. The prudent public health policy would be to pull [cigarette] marketing until they can prove it does no harm." Poole and Maclure agree. Maclure points out that in the 1980s some epidemiologists were skeptical that aspirin causes Reye's syndrome, but after then–Surgeon General C. Everett Koop issued a warning, drug companies changed their aspirin labeling. And, says Maclure, "hundreds of lives have been saved."

But Pierce is not hopeful that the tobacco companies will follow suit. Instead, he said, expect more advertising of the type the R.J. Reynolds Tobacco Co. has placed in the *New York Times* and other major newspapers: It argues that "the answer [to teenage smoking] isn't more bureaucracy ... [but] to teach young people how to resist peer pressure."

–Rachel Nowak

GENETICS

Rajewsky to Head EMBL's Italian Lab

LONDON—European plans to create a major new center for mouse genetics in Italy received a big boost this week when Klaus Rajewsky, one of Europe's most distinguished immunologists, agreed to head part of the venture. Rajewsky plans to divide his time between his current post at the University of Cologne's Institute for Genetics and a new program to be established in 1996 by the European Molecular Biology Laboratory (EMBL) at Monterotondo, 30 kilometers northeast of Rome. EMBL is establishing the program to help address Italian complaints that the country hasn't been getting sufficient return for its contribution to the organization.

INSTITUTE FOR GENETICS, UNIV. OF COLOGNE

Double duty. Klaus Rajewsky will divide his time between Monterotondo and Cologne.

Rajewsky, whose appointment was announced this week, will head three or four new research groups—funded by EMBL at up to $1.4 million per year—focusing on mouse genetics and the use of mouse mutants for understanding basic biological mechanisms and modeling human diseases. In a separate move, the European Union (EU) is also planning to establish a mouse repository at Monterotondo—the European Mouse Mutant Archive (EMMA)—and Italy's funding body, the Consiglio Nazionale delle Ricerche, is planning to relocate the Institute of Cell Biology from Rome and relevant research from other centers in Italy to the site.

"Rajewsky brings immediate credibility and recognition for Monterotondo which didn't exist before," says Peter Gruss, a developmental biologist at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany. "The three separate components planned for Monterotondo should create sufficient research density to make it a major European and worldwide center for mouse genetics, and with Rajewsky's appointment interest in it will soar," Gruss predicts.

Rajewsky's appointment is seen as a personal success for Fotis Kafatos, director-general of EMBL. Kafatos led efforts last year to keep Italy in EMBL after it threatened to quit, complaining that it was not getting enough back for its 16% contribution to the laboratory's $44 million annual budget. The threat was withdrawn late last year following EMBL's efforts to get some member countries more involved in the work of the laboratory. EMBL promised to establish the new genetics program at Monterotondo, alongside the EU's proposed EMMA facility. "Rajewsky has the seriousness and commitment for something quite new like the EMBL program," says Kafatos.

The Monterotondo campus was built as a biotechnology facility for the ENI company, which decided not to go ahead with its plans and made the laboratories available for academic use. "The buildings are very pleasant, and there's room for expansion," says Kafatos.

Rajewsky's own research interests dovetail neatly with the new center's agenda, and he will be establishing his own research team at the site. He and his team have pioneered techniques that allow researchers to remove genes precisely from specific cells of the body and at specific stages of development. Although Rajewsky initially developed the techniques to study antibody production, they are applicable in many fields of research, says Tak Mak, an immunologist at the Ontario Cancer Institute in Toronto. "The techniques are opening a new era of potentially much more sophisticated experiments," he says.

Although Rajewsky's new job does not involve EMMA directly, researchers hope that he will also lend credibility to the archive. EMMA will act as a collecting and distribution center for novel mouse mutants created by European researchers, on the lines of the Induced Mutant Resource at Jackson Laboratories in Maine. Rajewsky says most laboratories do not have the people and resources to test mouse mutants and distribute them to other laboratories on a large scale. "You need the professional skills of places like the Jackson Laboratories," he says. "A center like the Jackson Laboratories in Europe would be wonderful."

–Nigel Williams

Antisense Has Growing Pains

Efforts to develop antisense compounds as therapies for cancer, AIDS, and other diseases have encountered some unexpected questions about how the drugs really work

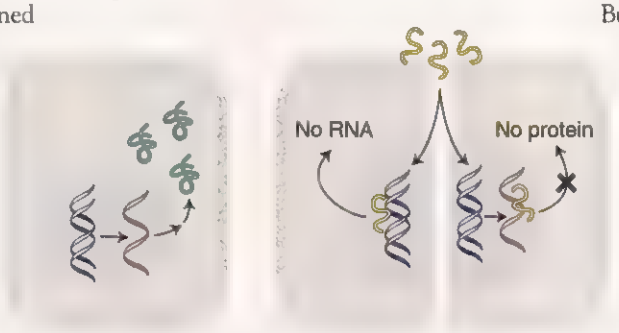
When *Science* named the gene-blocking technique known as antisense technology runner-up for its 1992 "Molecule of the Year," the accolade seemed well deserved. At the time, the technology appeared to offer a promising way to turn specific genes on or off at will. And that had made it potentially a powerful tool for uses ranging from fundamental molecular biology to the development of pharmaceuticals. Indeed, firms, both new and established, were rushing to exploit the technology to produce novel, rationally designed drugs for treating conditions ranging from genetic diseases to viral infections, including AIDS, and even cancer.

But during the past few years, the technique has run into unforeseen problems, and some of that early gloss has begun to wear off. Although several clinical trials have already begun, and there have been some promising results, researchers have encountered difficulties in getting antisense drugs—usually short pieces of DNA (called oligonucleotides) that have been designed to recognize and bind to specific genes—into target tissues. And potentially toxic side effects, including decreased blood clotting and cardiovascular problems such as increased blood pressure and decreased heart rate, have shown up in animal studies that have served as the basis for early human trials. But the biggest concern is that antisense compounds simply don't work the way researchers once thought they did.

"The assumption is that we are designing oligonucleotides that don't interact with anything besides [their targets]," says Cy Stein, an assistant professor of medicine and pharmacology at Columbia University's College of Physicians and Surgeons in New York City. "Many people are worried that a lot of the positive effects reported are not just antisense but other nonantisense mechanisms as well."

This uncertainty about what antisense drugs are doing inside the body has caused some experts in the field to argue that clinical trials have begun far too soon. "It is too early to take these things to human beings ... when we don't even know how they are working in a test tube," contends Ramaswamy Narayanan, who studies antisense drugs at Hoffmann-La Roche Inc. in New Jersey, but is not involved in any of the trials.

Others argue that even if the basic researchers haven't yet worked out the drugs' mechanisms of action, clinical trials are justified as long as the compounds show signs of efficacy. "As a clinician, what matters to me is if the drug works," says Jeffrey Holt, a pathologist at Vanderbilt University in Nashville, Tennessee, who is currently trying to use antisense DNA to fight advanced-stage breast cancer. "In medicine, people give drugs that we don't know the mechanism for." As one example, he cites aspirin, whose mode of action was not understood until relatively recently, even though it's been widely used for a century.



Holding on. In an untreated cell *(left)*, a gene's double-stranded DNA is transcribed into RNA, which then makes the protein *(green)*. Antisense drugs *(yellow)* are supposed to block this, by binding to the gene *(near right)* or the RNA *(far right)*. But do they?

Early promise

One reason antisense technology looked like the answer to drug designers' prayers is that it seemed to be simple and straightforward. During the first step of protein synthesis, in which genes are copied into RNA, only one strand of the double-helical DNA is so transcribed. The original idea, developed in the late 1970s and first published by Harvard Medical School researcher Paul Zamecnik, was to create a second RNA or DNA with a particular gene's complementary sequence—the so-called antisense molecule—that could recognize and bind to the RNA. This was supposed to prevent the RNA from manufacturing its protein, either directly or by causing it to be broken down by RNA-cutting enzymes. In the years since then, the technology has undergone several modifications, however.

To try and produce new drugs, researchers chemically string together a sequence of about 20 DNA bases—the oligonucleotide—that mirrors a short stretch of the gene scientists want to block. These may act either by binding to the RNAs, as the longer molecules do, or by binding directly to a target gene, thereby preventing it from being transcribed into RNA in the first place. (This latter approach is sometimes called "triplex" technology because a third DNA strand is being added to the two already in the DNA double helix.)

But however they work, such short oligonucleotides are much easier to synthesize than long antisense RNAs or DNAs. Researchers also made them more resistant to the many enzymes that break down nucleic acids by replacing a critical oxygen atom in each nucleotide building block with a sulfur atom. That's an important plus for a drug that has to be administered to a live human being, as it helps ensure that the drug will last long enough to do its job.

These modifications seemed to put drug designers on the right track: In initial tests with cultured cells, the sulfur-modified oligonucleotides, called phosphorothioates, appeared to work. For example, a team at Hybridon Inc., a biotech firm in Worcester, Massachusetts, found that one of their phosphorothioates, which they called GEM91, blocks replication of the AIDS virus, HIV-1, by targeting a viral life cycle gene called *gag*. "The antisense compound can suppress viral activity in vitro by up to 100%, depending on the concentration we use," says Sudhir Agrawal, vice president of drug discovery and chief scientific officer of the company. Other researchers also had early success in blocking reproduction of HIV-1 and other viruses with the sulfur-modified antisense constructs.

The successes quickly spurred the start-up of several biotech enterprises, such as Gilead Sciences Inc., an 8-year-old biotechnology company based in Foster City, California. "When we began, we said, 'Obviously from the literature, the technology works,' " recalls Richard Wagner, a molecular biologist at the company. "We thought that all we needed to do was bring in a few chemists and we were going to be rich."

But shortly after setting up shop, Gilead researchers realized it wouldn't be that simple. They quickly found that antisense

SOME CURRENT U.S. ANTISENSE CLINICAL TRIALS

Company	Disease	Rationale	Number of Patients
Isis Pharmaceuticals	CMV retinitis in AIDS patients	Block CMV reproduction	200+
Isis Pharmaceuticals	Genital warts	Block human papilloma-virus reproduction	70+
Isis Pharmaceuticals	Kidney transplant rejection	Block immune cell activities	20 to 40
Isis Pharmaceuticals	Rheumatoid arthritis and other autoimmune diseases	Block immune cell activities	20 to 40 per disease
Lynx Therapeutics	Chronic myelogenous leukemia	Block cancer gene activities	50+
Hybridon	AIDS	Block HIV reproduction	125

compounds applied to a strain of human blood cells did not even get into the nucleus, the site of their RNA or DNA targets, Wagner explains. To get around that problem, they were forced to inject the compounds directly into the cells, a technique that works well in laboratories but cannot be applied to patients.

They did get some encouraging results, though: When they performed the injections, Gilead workers found that compounds directed at the *rev* or *gag* genes located in HIV-1 inhibited viral replication in the cells. In other experiments, antisense oligonucleotides targeted to the *c-myc* gene of blood cells from leukemic patients shut down cancer cell proliferation. But in both sets of experiments, yet another glitch cropped up.

To their surprise, researchers found that oligonucleotides they were using as controls, which couldn't recognize the *rev*, *gag*, or *c-myc* genes, either shut down virus replication or blocked cell proliferation almost as effectively as the ones they were testing as drugs. "While we could repeat many of the biological effects caused in cell culture, in every case our controls would show the same response," Wagner notes. "When we went back to the original papers, we found that often these controls were missing."

At first, Gilead researchers kept their concerns quiet. "There were a significant number of people claiming that these things worked," Wagner explains. "We really didn't want to go public with our negative results until we were sure that we weren't doing something wrong in our system." By the early 1990s, however, other researchers were echoing Wagner's concerns.

One example comes from Arthur Krieg of the University of Iowa, Iowa City, and his colleagues, who were attempting to develop antisense compounds that could be used to treat autoimmune diseases, such as rheumatoid arthritis, in which the immune system begins attacking the body's own tissues. "The B cells in autoimmune disease are hyperactive," Krieg explains. "We were trying to identify the genes responsible and shut them down."

When the researchers tried to inhibit B cells in culture with antisense DNA, however, the molecules turned B cell function up instead of down. That result was a mixed blessing, because it suggested that while the compounds tested would not be useful for treating autoimmune diseases, they might help buttress immune cell function in AIDS patients. But the Iowa team encountered an anomaly in their system similar to the one the Gilead workers had previously found. "Later, we got concerned as a number of controls also turned out to be B cell activators as well," Krieg recalls.

The immunologist, who says he worked "full time" to figure out what was causing this, came up with a solution earlier this year. In a paper published in the 6 April issue of *Nature*, Krieg and his colleagues reported evidence suggesting that antisense oligonucleotides mimic bacterial DNA in triggering a potent response by mammalian immune cells. They based this conclusion on experiments in which they showed that DNA fragments containing the two-base sequence CpG (where C stands for the nucleotide base cytosine, the G for guanine, and p for phosphate) activate mammalian B cells and natural killer cells in culture.

This only takes place, however, when the CpG motif lacks methyl groups. Because such sequences are common in bacterial DNA, but not in mammalian DNA, where most nucleotides have an attached methyl group, the immune response may be a way of defending against bacterial infections, Krieg suggests. The finding applies to antisense technology because antisense manufacturers don't usually add methyl groups to their synthetic oligonucleotides. Thus, mammalian immune systems that encounter such compounds with the CpG motif may be tricked into thinking they have been invaded by bacterial aliens and consequently spring into action.

Krieg suggests that this response could be useful clinically, but he says researchers need to be aware that the drugs are working directly on the immune system, rather than, say, targeting the AIDS virus itself. "I am firmly convinced that synthetic oligonucleotides, like the ones in clinical trials now, will make useful drugs for human beings," Krieg says. "But I don't think they are working through true antisense mechanisms."

Side effects in animals

Besides not always working by "true antisense mechanisms," the synthetic oligonucleotides have also caused side effects in experimental animals. When administered by one-time injection in high doses to monkeys, for example, several phosphorothioate drugs were lethal to some of the animals, for reasons that are not yet understood. In others, the oligonucleotides caused a transient decrease in the total number of two kinds of white blood cells as well as changes in blood pressure and heart rate, according to Hybridon's Agrawal. In addition, phosphorothioates have been found by Hybridon and Isis researchers to accumulate in the liver, kidneys, and bone marrow of animals, although the long-term effects of this deposition are not clear.

Some of these effects may be explained by the drugs' propensity to bind to proteins, says Columbia's Stein. At a recent meeting on the "Art of Antisense,"* molecular pharmacologist Stein presented some of his teams' findings on why the compounds often don't make it to the nucleus. They've found that they end up instead in the endosomes, small membrane-bound vesicles in the cytoplasm. This apparently occurs because the oligonucleotides tend to bind to proteins, which are themselves incorporated in the endosomes. "Many cell types protect themselves by sequestering oligos in intracellular compartments," Stein says, but this could also contribute to the deposition of the drugs in liver and kidney.

In addition to getting entangled by proteins inside cells, the Columbia researcher found that many synthetic oligonucleotides, because of their highly negative charge, get hung up on proteins outside cells as well. Among these are growth factors and cell anchoring proteins such as fibronectin and laminin. The result is that antisense compounds block cell migration and adhesion to underlying tissue in vitro—an effect that may interfere with wound healing and arterial wall repair in living animals, Stein says.

Hybridon's Agrawal maintains, however, that the cardiovascular and other effects seen in animals can be minimized in patients by using low doses of the compounds and administering them gradually by continuous intravenous injection. That seems to be borne out by the early results of Hybridon's

* The meeting, which was sponsored by *Nature Medicine*, was held in New Orleans on 21 and 22 September.

clinical trial of GEM91 in AIDS patients, he told participants in the antisense meeting. Agrawal also reported that patients getting the higher doses are showing signs of clinical improvement in that their viral counts drop a few days after the treatments, although it is far too soon to tell whether this translates into improved survival. To Agrawal, it doesn't matter how the drugs work, if they end up helping AIDS patients. "Despite all the other properties [in addition to actual gene targeting], we feel that if we find an antisense effect ... then we have a new drug," Agrawal says.

Looking to the future

Agrawal is not the only one who hasn't lost faith in the technology. Biotechnology representatives argue that the problems turning up with antisense oligonucleotides are common in drug development, especially when untested, new technologies are being explored. "Every new technology starts at the bottom, in essence, getting your foot in the door," says Gerald Zon, vice president of medicinal chemistry at Lynx Therapeutics Inc., a biotech company in Hayward, California. He notes that every new drug has negative effects that must be weighed against clinical benefits. The answer, he says, is to design better second- and third-generation drugs in order to boost drug efficacy while, at the same time, minimizing unwanted side effects.

Indeed, researchers at companies such as Hybridon, Isis, and Gilead say they are applying the lessons they are learning from the animal studies and early clinical trials to try to come up with better and less toxic compounds. The options they are exploring include modifying the structures of oligonucleotides so that they bind less readily to proteins or more readily to their target genes. All three companies are also generating fat-soluble delivery molecules called cationic liposomes. The researchers hope these lipid-loving shuttles will help antisense compounds break through cellular barriers that prevent entrance into the nucleus.

These new compounds and delivery systems carry no guarantees that they will be any better than the phosphorothioates used in the current clinical trials. But even some of the critics, such as Stein, agree the field still holds great promise, as long as the researchers recognize that antisense drugs don't always work the way they are supposed to. "My guess is that we will find that the current generation of phosphorothioates are extremely active biological molecules," Stein concludes, "and that they work by many mechanisms, of which antisense is one. The truth is that we'll have to wait and see. None of us really knows what is going to come out of it."

–Trisha Gura

Trisha Gura is a reporter on leave from the Chicago Tribune.

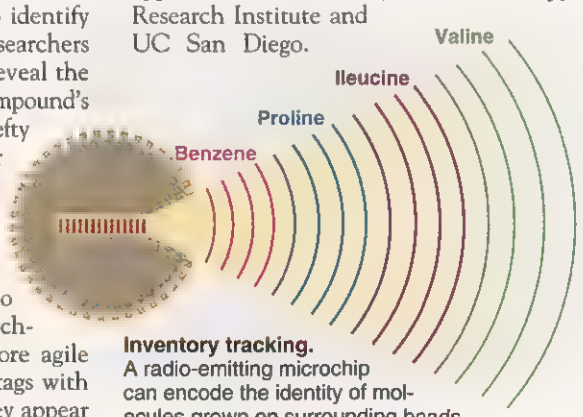
CHEMISTRY

Radio Tags Speed Compound Synthesis

Like aging computers, it doesn't take long for scientific techniques to seem slow and cumbersome. Take combinatorial chemistry. When it was introduced a few years ago, it was the supercomputer of chemical synthesis. The technique allows chemists to quickly paste together several different chemical building blocks into millions of combinations, in hopes that one will prove to be a new drug or a useful material. To identify each one of the new compounds, researchers typically affix chemical tags that reveal the unique arrangement of each compound's components. But these tags carry a hefty price: Their use doubles the number of chemical steps—and the time—involved in the assembly process, and their fragility prevents the synthesis of some compounds.

In the past 2 weeks, however, two separate groups of California researchers have unveiled a faster and more agile model. By replacing chemical ID tags with tiny radio-emitting microchips, they appear to have overcome both of the problems inherent in the old one. "The upshot is that it makes the whole process of drug discovery more efficient," says Rob Armstrong, a chemist at the University of California (UC), Los Angeles, who led one of the research groups, which includes scientists from Ontogen Corp. in Carlsbad, California. "This has the potential to be a significant advance in simplifying the encoding process," adds Michael Pavia, who heads combinatorial research at Sphinx Pharmaceuticals in Cambridge, Massachusetts. The technique not only saves time, says Pavia, "it gives you a wider range of chemical diversity to select from in building your new molecules."

Armstrong's group presented its findings at last week's meeting of the Western Biotech Conference in San Diego, as did the second team, led by Michael Nova at IRORI Quantum Microchemistry in La Jolla, California, and K. C. Nicolaou, who holds dual appointments at the La Jolla–based Scripps Research Institute and UC San Diego.



Inventory tracking. A radio-emitting microchip can encode the identity of molecules grown on surrounding beads.

The IRORI group was, however, the first in print, with a paper in the 15 October issue of *Angewandte Chemie*.

Both techniques add considerable power to combinatorial chemistry, which already made traditional synthetic chemistry look like an old IBM punch card. Traditionally, novel compounds are synthesized one at a time, but combinatorial chemists create huge numbers in a single process by assembling a few chemical building blocks—each of which has a corresponding ID tag, such as a short nucleotide sequence—in all possible combinations. Chemists need these tags to decipher the makeup of compounds that show promise in an assay, such as the ability to kill cancer cells (*Science*, 3 June 1994, p. 1399).

But because a tag has to be added with each building block, assembling a 10-component molecule actually involves at least 20 time-consuming chemical steps. And the technique runs into trouble when creating small organic molecules, which constitute most of today's drugs. Some of the synthetic reactions involve potent reagents, such as hydrofluoric acid, which can rip ID tags apart.

The new microchip tags appear to solve both these problems at once. A chip, which emits a binary code, is inserted into a mesh capsule loaded with polymer beads—the "seeds" to which combinatorial building blocks are added by dunking the capsule in a series of beakers. In the Ontogen approach, a nearby radio scanner registers both the identity of a capsule and the contents of each beaker it enters. These data are uploaded to a computer that keeps track of the order of building blocks in the growing molecule. In the IRORI approach, the information is stored on the microchip itself, using a transmitter that writes the information to the chip. The information is uploaded to the computer only when the assembly run is complete.

By eliminating the chemical tags, both approaches do away with half the synthetic steps involved, yet end up with an instantly available computer record of the precise structure of the compounds in each capsule. Moreover, says Nicolaou, "now you are free to use any chemistry you want to build your molecules." Speed and flexibility—for chemists, it's a winning combination.

–Robert F. Service

Defining the First Steps on the Path Toward Cell Specialization

When poet Robert Frost's two roads diverged in a yellow wood, he regretted that he "could not travel both/and be one traveler." But a cell that reaches such a fork in its developmental path isn't forced to choose between one road or the other: It can become two travelers, by dividing into two cells with different fates. This maneuver is the basis for complex life, as a single cell splits into cells with distinct functions, eventually becoming part of specialized tissues and organs. Yet the mechanisms for the initial, asymmetric division—how different genes are activated in each progeny cell—have mystified developmental biologists for decades.

Now a team of researchers in Paris and Cambridge, Massachusetts, has—in a simple model, a bacterium—traced this mechanism back to a single protein. The researchers focused on a phenomenon called sporulation, in which certain bacteria divide to produce two distinct progeny that have identical sets of genes yet take very different paths: a "mother" cell and a "forespore" that is biochemically specialized to become a dormant spore. On pages 637 and 641 of this issue, the investigators report that near the very beginning of this type of cell division in the bacterium *Bacillus subtilis*, a single protein called SpoIIE initiates a cascade of biochemical changes on one side of the burgeoning cell membrane, leading to the activation of scores of genes that convert the forespore into a spore. And this activity may be tied to the size of the nascent cell.

The achievement—by a group led by molecular geneticist Patrick Stragier of the Institut de Biologie Physico-Chimique and developmental biologist Richard Losick of Harvard University—has triggered a chorus of praise from other researchers. "Losick, Stragier, and colleagues have relentlessly and elegantly kept moving the problem of asymmetry back to earlier and earlier stages. They now have a strong suspect for a molecule that may well be what makes the two cells different," says Ira Herskowitz, a developmental geneticist at the University of California, San Francisco (UCSF). Adds David Kirk, a developmental geneticist at Washington University in St. Louis, "It's beautiful work."

Kirk and other researchers have found clues to the relationship between cell size and cell fate in *Volvox carteri*, a species of algae, and to the way signaling proteins are restricted to specific progeny cells in the developing nervous system of the fruit fly *Drosophila melanogaster*. But, says Alan Grossman, a molecular geneticist at the Massachusetts Institute of Technology (MIT), "No other model system has gotten as close as *B. subtilis*." Researchers caution that the Losick-Stragier team still hasn't pinned down the reasons for one crucial initial step, when a membrane called the septum forms near one end of the parent cell. But investigators in their labs, at Oxford University in England, and at other institutions are hot on the trail of this event.

Together, all these projects signal that cell fate studies themselves may be entering a crucial period of discovery. Evolution's inherent economy means that such mechanisms may be conserved in other simple organisms, and perhaps even in higher ones, such as mice and humans. "Mechanisms aren't going to be transferred lock, stock, and barrel across organisms," says Michael Yudkin, a biochemist at Oxford. "But any mechanism discovered in one organism is helpful in inspiring experiments in another."

C. WEBB

Division street. The protein SpoIIE *(green band)* collects at the site of cell division in these bacteria (each cell=2 microns), triggering different patterns of gene activation on either side.

Divide and differ. The findings by the Losick-Stragier team cap decades of study of the ubiquitous *B. subtilis*, which can be readily found in almost any handful of dirt. Sometimes, however, nutrients in the soil are not nearly as abundant as the bacteria. When food is scarce, a *B. subtilis* cell stops reproducing through normal, binary fission, and instead duplicates its chromosomes and partitions them into two contiguous but differently sized cells, the mother cell and the smaller forespore. The mother cell expands and engulfs the forespore, coating it with layers of protective proteins, then dies. With its genetic material bound up in a sturdy, inactive mass, the armored spore can survive starvation in a state of suspended animation for decades.

Because the mother cell and the forespore are genetically indistinguishable, spore formation must depend on the activation of distinct genes in each cell. Experiments in Losick's lab in the late 1960s led researchers to suspect that differential activation is accomplished by a series of transcription factors (proteins that bind to sites near the beginning of genes to initiate messenger RNA synthesis) called σ factors. By 1991, Losick and colleagues had identified one such factor, σ^F, that is active only in the forespore (*Science*, 25 October 1991, p. 562). Says Losick, "It became clear that if we wanted to understand how cell-specific gene expression begins, we'd have to look at the mechanisms that restrict σ^F activity to the forespore."

One promising candidate for this mechanism was the protein SpoIIE. Losick's group found that mutant bacteria lacking the protein also lacked σ^F activity. Eager to see where SpoIIE was acting in living bacteria undergoing sporulation, the group starved the bacteria to induce spore formation and labeled the protein with fluorescent antibodies or a green fluorescent protein. In the first of their two papers, Scott Alper, Fabrizio Arigoni, Leonard Duncan, Kit Pogliano, Chris D. Webb, Losick, and Stragier demonstrate that SpoIIE collects exclusively at the developing membrane between mother cell and forespore. Says Losick, "It's just the perfect vantage point to somehow activate the transcription factor on one side of the division but not the other."

Yet that "somehow" was troubling. The group "still didn't know what SpoIIE was doing biochemically," Losick says. Then, last year, they defined the likely functions of two other players in the σ^F regulation game: the "anti-σ" protein SpoIIAB, which binds to σ^F and thus blocks its activity; and the "anti-anti σ" protein SpoIIAA, which inhibits SpoIIAB's binding action. Alper, Duncan, and Losick showed, in test-tube experiments, that the three substances interact in a partner-switching process. SpoIIAA binds to SpoIIAB, forcing the latter to give

up any bound σ^F, freeing the transcription factor—in theory—to activate genes (*Cell*, vol. 77, p. 195, 1994).

But SpoIIAA can't bind to SpoIIAB—and release σ^F—if it's carrying a phosphate group, and that's where SpoIIE seems to come into play. A group led by Yudkin and molecular geneticist Jeffrey Errington at Oxford had shown this phosphate-driven inhibition of SpoIIAA-SpoIIAB binding—without discerning its exact consequences for σ^F—in 1993 (*Cell*, vol. 74, p. 735, 1993). That meant, Duncan says, that "if a pool of unphosphorylated SpoIIAA is required to generate free σ^F, there had to be a phosphatase" at the beginning of the cascade that was capable of dephosphorylating SpoIIAA. "It occurred to us that SpoIIE could be the phosphatase."

Indeed it is. In in vitro studies, phosphorylated SpoIIAA exposed to purified SpoIIE lost its phosphate group. Further experiments showed that when a single amino acid residue, the binding site for the phosphate group, was changed on SpoIIAA, SpoIIE failed to dephosphorylate it—suggesting that, in their nonmutant forms, the two proteins are tailored specifically to interact with each other in a pathway for the release of σ^F. Comments Yudkin, "I think the results are robust. Everybody's views are moving toward convergence" on the importance of phosphorylation reactions in σ^F's release.

A matter of concentration? This release is triggered in the forespore, and not the mother cell, because SpoIIE is more concentrated in the former, the researchers think. "The answer may be that SpoIIE goes to the septum," says Stragier. He points out that because the forespore has a smaller volume, the molecules are crammed into less space, and that means more phosphorylated SpoIIAA comes into contact with SpoIIE. Comparatively more dephosphorylation reactions occur, leading to the release of more σ^F. The researchers are currently testing this theory in vivo.

The next big question, of course, is how parent cells form a septum at one end to produce progeny cells of unequal sizes. During binary fission in *B. subtilis* and several other bacteria, a cytoskeletal protein called FtsZ forms a contracting ring at the dividing cell's midpoint. Errington suggests that the same process probably occurs near one pole during sporulation, but only after utilization of a central "marker" for cell division is somehow blocked. "The problem is, we don't even understand how the cell can choose [to divide at] the central position" during normal cell division, Errington says. Losick's group suggests that sporulation induces a chemical "switch" that frees FtsZ to assemble at other markers closer to the poles. But "there's still no sense of what might be attracting [FtsZ]" to these new markers, Losick says.

B. subtilis isn't the only species in which relative cell size may make a developmental difference. Developing embryos of the green alga *Volvox carteri*, for example, have identical cells through the first five rounds of cleavage. But these cells eventually develop into two distinct kinds: small, nonreproducing somatic cells and much larger "gonidia" or reproductive cells. It's in the sixth round of cleavage that some embryonic cells begin dividing asymmetrically, producing both small and large progeny. All cells under 6 microns in diameter develop as somatic cells, Kirk and his colleagues at Washington University have found, while those over 9 microns develop as gonidia (*Journal of Cell Biology*, vol. 123, p. 191, 1993).

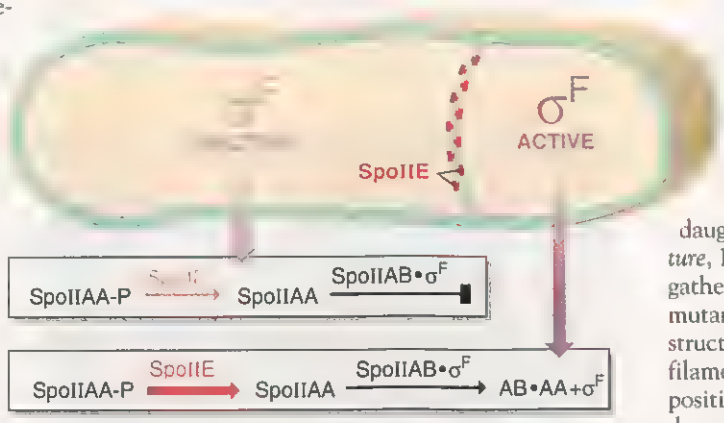
Kirk suggests that the difference in cell fates may be partly dictated by the ratio of chloroplasts to nucleus in each cell. Because the number of chloroplasts in each *Volvox* cell grows with increasing cell size, but each cell has only one nucleus, that ratio is naturally higher in larger cells. The abundance of chloroplast proteins entering the nucleus might therefore be higher as well, Kirk says. And while many of these proteins coordinate the transcription of nuclear genes essential for photosynthesis, he speculates that some could play roles in the transcription or repression of genes that specify somatic and gonidial cell fate.

Tracing the simple and the complex. Asymmetric cell division is also giving up some of its secrets in the fruit fly *Drosophila melanogaster*. The two nerve cells and two support cells that make up an organ called an external sensory bristle, part of the fly's peripheral nervous system, all develop from a single precursor cell. The progeny cell that will later divide to become the nerve cells receives the precursor cell's entire complement of a membrane-associated protein called Numb and a transcription factor called Prospero, both believed to be involved in the determination of cell fate. But how these substances are segregated only to the preneural cell remained unknown—until recently.

UCSF geneticists Jürgen Knoblich, Lily Jan, and Yuh Nung Jan reported in last week's issue of *Nature* that during mitosis Numb and Prospero collect in a crescent-shaped spot overlying one of the two centrosomes, organizers of the mitotic spindle that pulls separated daughter chromatids to the cell poles (*Nature*, 19 October 1995, p. 624). The proteins gather on one side of the dividing cell even in mutant cells with extra centrosomes or when structures such as microtubules and actin filaments are destroyed, suggesting that their positioning is coordinated with—but not dependent on—the cytoskeletal changes characteristic of mitosis. Knoblich thus speculates that a "master organizer" provides positional information for both spindle orientation and the Numb-Prospero crescent. "The key point is, if the cell wants to divide asymmetrically, there is machinery at hand to segregate proteins into one of the two daughter cells," Knoblich says.

As other studies of asymmetric cell division move ahead in the bacterium *Caulobacter crescentus*, the budding yeast *Saccharomyces cerevisiae*, the nematode *Caenorhabditis elegans*, and other organisms, scientists hope that even complex problems such as tissue specialization in mammalian embryos may eventually be better understood. The differential activation of identical genomes segregated to two progeny cells "is an absolutely central mechanism in biology, and one of enormous importance," says Yudkin. And while it's a big leap from bacteria, algae, and flies to mammals, tactics of asymmetric division such as protein phosphorylation and localization are likely to be used over and over, he and others say. Says MIT's Horvitz: "The biochemical data, although it may seem like organism-specific nitty-gritty, really tends not to be. We have to understand the minutiae in order to know the big picture." In other words, the details are what make all the difference.

—Wade Roush



Cramped quarters. In one view of bacterial spore formation, SpoIIE contacts SpoIIAA more often in the forespore *(right)*, removing a phosphate group. This allows AA to bind to SpoIIAB, freeing σ^F—which activates genes—from AB's grasp.

Additional Reading

H. Robert Horvitz and Ira Herskowitz, "Mechanisms of Asymmetric Cell Division: Two Bs or Not Two Bs, That Is the Question," *Cell* **68**, 237 (1992).

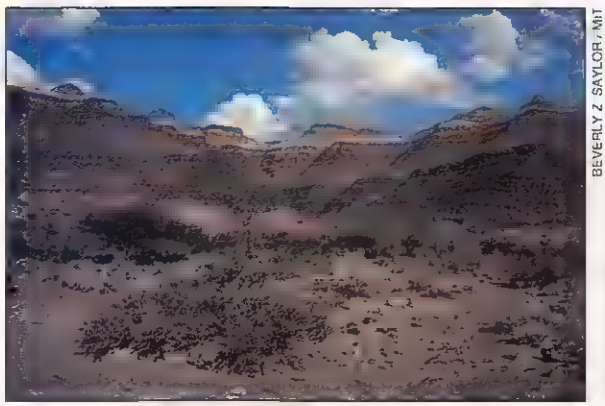
Lucille Shapiro, "Protein Localization and Asymmetry in the Bacterial Cell," *Cell* **73**, 841 (1993).

Animal Oddballs Brought Into the Ancestral Fold?

Life can take on odd forms, as any *National Geographic* program illustrates. But the oddest animals of all go back in time—way back. These are the Ediacara, frond- or disc-shaped blobs of living matter that inhabited Earth millions of years before the Cambrian Period, more than 540 million years ago. So strange are these creatures—a few ranged up to a meter in size but lacked an obvious mouth or anus—that their very nature remains in dispute 50 years after their discovery. Some paleontologists think that the Ediacara may be a part of the evolutionary path leading to later animal life. Others argue that they couldn't be: They believe the strange creatures were just a failed experiment in how to make an animal that ended before the Cambrian Period's explosion of evolutionary innovation—which created the basic animal shapes we see today—even got started. But now, excluding the Ediacara from the evolutionary chain of animal life has just gotten harder.

On page 598 of this issue of *Science*, geologist John Grotzinger and geochronologist Samuel Bowring of the Massachusetts Institute of Technology (MIT) and their colleagues describe new evidence indicating that instead of disappearing tens of millions of years before the Cambrian explosion, the Ediacara persisted at least up to the beginning of the Cambrian, if not into it. If so, they were around during the early stages of the Cambrian explosion and may have contributed to it. This conclusion, which is based on the researchers' dating of tiny mineral grains from volcanic ash layers interspersed among the Ediacaran fossils, is already winning plaudits from paleontologists. "If Grotzinger and company are correct, that's excellent news," says Cambrian paleontologist Simon Conway Morris of the University of Cambridge, in the United Kingdom. "It's not easy to trace simple connections between the Ediacaran and Cambrian fossils, yet if they are effectively cheek by jowl, then it's got to focus our attention on a proper understanding of the Ediacaran fossils."

Paleontologists have been trying to understand the Ediacaran fossils since they were first found in the Ediacara Hills of southern Australia in 1946. The flat, often quilted-looking fossils have been called almost every name in the book. Because the fossils show no obvious signs of characteristic features of higher animals, such as circulatory and digestive systems, Adolf Seilacher of Tübingen University in Germany has argued that the Ediacara were enormous single cells. That would warrant their classification into a kingdom of their own, a kingdom that died out when the Ediacara fossils disappeared from the geologic record, apparently well before the Cambrian.



BEVERLY Z. SAYLOR / MIT

Ediacaran heaven. Southern Namibia's rugged terrain has proven an ideal place to search for animals' early ancestors.

Paleontologist Gregory Retallack of the University of Oregon agrees that Ediacarans weren't animals, but he thinks they were primitive lichens—symbiotic combinations of algae and fungi. Examining some Ediacaran fossils, Retallack concludes that the original organism resisted compression after burial about as well as sturdy tree ferns, making the Ediacara far tougher and less compressible than the jellyfish and worms they have been compared to. Lichens would meet that standard of toughness as well as feed themselves without circulatory or digestive systems.

Then there are the traditionalists, such as Conway Morris. They argue that many, although not necessarily all, of the Ediacara fossils strongly resemble animals that follow, such as corals, jellyfish, segmented worms, and arthropods. This is evidence, they believe, that Ediacara gave rise to at least some of the animals seen today. But their line of reasoning had a big a hole in it: "A consensus had begun to emerge," says Conway Morris, "that there's some sort of gap" between the last of the Ediacara and the first of the Cambrian animals. The youngest Ediacaran fossils ever found still seemed older than the oldest Cambrian fossils. There was even talk of their dying out in a Precambrian mass extinction.

BRUCE RUNNEGAR/UCLA

A dead end? Was this Ediacaran any animal's ancestor?

Enter Grotzinger, Bowring, geologist Beverly Saylor of MIT, and geochemist Jay Kaufman of Harvard University. To get a fix on when the Ediacara died out, they went to rock outcrops in southern Namibia that hold a rich load of Ediacaran fossils. Linking those or any other Ediacaran fossils found around the world to fossils in Cambrian rocks has been difficult because any one outcrop exposes only a short segment of the late Precambrian and Cambrian record. So, to determine whether a particular rock layer's fossils are younger or older than another's, researchers had to try to tie together outcrops that are many kilometers—even thousands of kilometers—apart. Such relative dating has recently hinted that the Ediacara approached or even overlapped the Cambrian fauna. But the relative dates cannot approach the reassuring directness of absolute dates, and that is what the new work provides.

Geochronologist Bowring determined the absolute age of beds of volcanic ash by measuring the amount of lead produced in zircon mineral grains through the radioactive decay of uranium. The uranium-lead method has been refined in recent years to the point that even rocks a half-billion years old can be dated with a precision of a million years or less. And when the MIT team applied the method to the Namibian rocks, they uncovered several surprises.

The period of highest Ediacaran diversity lasted not tens of millions of years, as previously estimated, but just 6 million years. And that 6 million years carried the Ediacara right up to the Precambrian-Cambrian boundary in southern Namibia. The youngest Ediacaran fossils found there were laid down just after an ash bed dated at 543.3 ± 1 million years ago; Bowring had already dated the Precambrian-Cambrian boundary in Siberia at 543.9 million years in the course of squeezing the Cambrian explosion down to less than 8 million years (*Science*, 3 September 1993, p. 1274).

As an added bonus, the dates on the four ash beds that span the 6 million years of high Ediacaran diversity came out in the same chronological order as predicted by the geological

ordering of the beds. That supports both the geological analysis and the accuracy of the dating, says Bowring. "It used to be that plus or minus 5 million years was great. Now the question is: Can we split hairs at the 200,000- or 300,000-year level?" Bowring intends to find out by dating more ash beds in Namibia.

The new high-precision dating argues against a gap that would keep the Ediacara from contributing to animal evolution in the Cambrian, but Grotzinger and his colleagues concede that their new dating does not rule out most of the proposed scenarios. The Ediacara could have continued to evolve into more familiar animals; they could have perished at or very near the boundary and not contributed anything to later evolution; or they could have been a sister group of the Cambrian animals, as Seilacher is now suggesting, sharing a common ancestor that was not preserved in the fossil record. The MIT-Harvard group's discovery of Ediacaran fossils in some of the youngest strata in southern Namibia, younger than any Ediacarans known elsewhere, is a reminder of how spotty the ancient fossil record can be. More fossil collection is needed to resolve the Ediacarans' role, paleontologists say. Namibia, they add, looks like a good place to start.

–Richard A. Kerr

NEUROBIOLOGY

New Clue to Brain Wiring Mystery

The most complicated wiring task in the world occurs right inside our heads. During brain development, many billions of neurons must make precisely the right connections for our brains to work as they should. Developmental neurobiologists have learned in recent years that both the electrical activity of neurons and the presence of neuron-nurturing proteins called neurotrophic factors appear to be key to the final sculpting of neural connections. But they have not been able to figure out just what characteristics allow neurons to respond to those nurturing proteins.

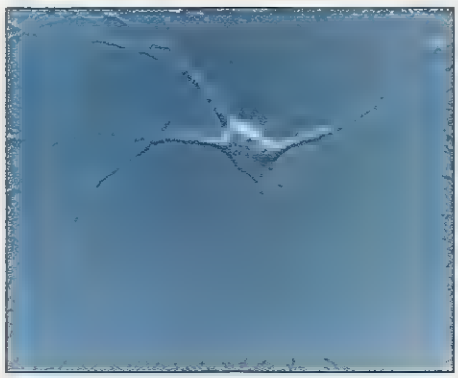
Now, in a paper in the October issue of *Neuron*, Barbara Barres and her colleagues at Stanford University School of Medicine provide a clue. In their studies of the survival of neurons in culture dishes, they discovered that pure preparations of neurons from rats' eyes must be in an activated state to be susceptible to the neurotrophic factors' effects.

The paper provides a "missing link," by connecting neural activity and neurotrophic factors, says Washington University neurobiologist William Snider. "It is a striking result," adds developmental neurobiologist Carla Shatz, of the University of California (UC), Berkeley, whose own work has implicated neurotrophic factors in the activity-dependent wiring of the visual cortex. The revelation that active neurons respond better to neurotrophic factors, she says, "may help interpret a lot of [other] results."

Barres's team was not directly addressing the problem of brain wiring, but rather was trying to determine the best conditions for growing cultures of purified retinal ganglion neurons, which in the developing embryo send their axons from the retina of the eye along the optic nerve to the brain. Central nervous system neurons such as these are notorious for dying when they are maintained in culture for any length of time, and sure enough, the retinal neurons died even though the researchers fed them an elaborate cocktail of trophic factors that they would be expected to encounter en route to the brain.

They tried stimulating the neurons, because other groups had shown that electrically activated nerve cells are more likely to survive in culture. That alone didn't do the trick, but when the researchers combined activation and trophic factors, the neurons at last survived. Apparently, activity raised levels of the intracellular signaling molecule, cyclic AMP, and that somehow enabled the neurons to respond to the trophic factors. Earlier studies had suggested activity may play such a role, but Barres is the first to verify it with pure cultures of neurons.

That finding caught the interest of developmental neurobiologists who study the remodeling that occurs during brain development. Developing neurons in the brain first make somewhat imprecise links with other neurons that must later be rearranged to create the specific wiring patterns needed for the brain to carry out its numerous functions. In the past decade, work from many research teams has shown that neurons whose electrical signals arrive simultaneously at a spot in the brain will add more connections in that area, while neurons that are inactive when others in the area are active tend to lose the connections they've already made. And in the past year, several groups have shown that neurotrophic factors may also play a role in this activity-dependent remodeling (see Article by Hans Thoenen on p. 593).



B. BARRES/*NEURON*

Survivor. Retinal ganglion neurons, such as this one, can live in culture when appropriately stimulated.

Now the Barres team has shown—at least in the culture dish—that it is the electrical activity itself that is the key to the selective effect of the neurotrophic factors. And these findings "fit together as a nice story" with another neuron culture study published last year, says UC San Francisco developmental neurobiologist Michael Stryker. In that study, Harvard University neurobiologist Michael Greenberg and postdoc Anirvan Ghosh showed that cultured neurons from the cerebral cortex of embryonic rats produce more of the neurotrophic factor BDNF when they are electrically active, and that the BDNF in turn enhances the cells' survival in culture (*Science*, 18 March 1994, p. 1618). But, says Ghosh, who's now at Johns Hopkins University, when the researchers simply added BDNF to the cultured neurons without stimulating them, it did little to help the neurons survive. Barres's work suggests that "not only did the cell need the BDNF it was making," says Ghosh, "but it actually needed to be in the [activated] state" to respond to the BDNF.

Together those papers suggest a model, says Stryker, in which a neuron receiving a signal produces more neurotrophic factor, and that factor in turn has a growth-promoting effect on nearby neural connections that are active at the same time.

While intrigued by the Barres paper, many neurobiologists caution that going from studies of neuron survival in a culture dish to predictions about synapse formation in a developing brain is a major conceptual leap that may not be justified by the Barres results alone. But despite his caution, Larry Katz of Duke University says his "gut feeling" is that the hypothesis will turn out to be right. Indeed, preliminary work, which will be presented next month at the annual meeting of the Society for Neuroscience by members of his lab and that of his Duke colleague Don Lo, shows that blocking the activity of neurons in slices of rat cerebral cortex blocks the growth-inducing effects of neurotrophic factors on those neurons. And that is just one of many related findings that are in the works in a number of labs, says Katz.

If these upcoming results continue to support the conceptual leap from the culture dish to the developing brain, researchers will be a bit closer to understanding how nature has solved the toughest wiring problem around.

–Marcia Barinaga

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GENOMESYSTEMS

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edited by JOCELYN KAISER

Aussie Rabbit Virus Causes Ruckus

Australian scientists are struggling to rid their country of a plague of alien rabbits that have wrecked the ecosystem and cause $100 million in farming losses each year. To begin restoring the balance, they hoped to deploy a virus to kill rabbits in a limited range. But in field tests on an island this month, the virus temporarily escaped, thanks to an unforeseen vector—bushflies.

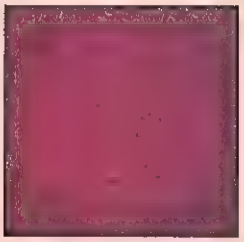
Wild European rabbits have overrun Australia's natural, marsupial-laden ecosystem with one dominated by rabbits, cats, and foxes. A myxoma virus was tried starting in 1950 as a control scheme, but it didn't always work. Rabbit calicivirus disease (RCD), from China, was the next great hope. RCD kills in 36 hours, so it seemed ideal for limited, quick-kill strikes. Such strikes were desirable because cats and foxes might further decimate native species if their staple diet—rabbits—was removed too rapidly.

Scientists with the Commonwealth Scientific and Industrial Research Organisation released the virus among rabbits in a quarantine enclosure on Wardang Island off South Australia last March. At first nothing much happened, but as the weather turned warm in early October, the virus killed 80% of the animals within a few days.

Then the experiment started going awry. Virus-killed rabbits turned up outside the enclosure, and two soon were found on the adjacent mainland near Point Pearce. Scientists now suspect bushflies, says project director Nicholas Newlands. "We noticed ... there were bushflies active in the warrens back in April," he says. The flies may have fed on sick rabbits in the enclosure, then crossed 5 kilometers of sea to infect mainland rabbits.

After a frenetic week destroying rabbits on the island and around Point Pearce, scientists think they have doused the epidemic, although some conservationists are still concerned. Australian officials want further public discussion before RCD is unleashed on the mainland. The virus could prove more potent than a limited strike weapon, because in spring and summer, bushflies are thicker than rabbits in Australia.

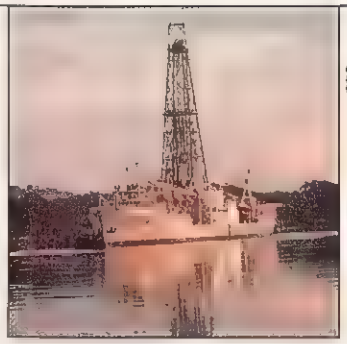
GONG Show



This image, showing the velocities of sound waves crossing the surface of the sun, is a glimpse of things to come from a worldwide network of solar detectors, switched on this month.

The Global Oscillation Network Group (GONG)—six observation stations in California, Hawaii, Australia, India, the Canary Islands, and Chile—is measuring these velocities every 60 seconds. The waves are thought to be caused when boiling gases create vibrations that propagate through the sun, causing it to "ring" like a bell. Comparisons of oscillation frequencies of waves passing through different portions of the sphere will yield details on the sun's internal temperature, interior motion, and chemical composition, much as seismic waves on Earth reveal the density and composition of our planet.

Previously, scientists have followed these oscillations for short periods as the sun passed over individual sites, but the global network will produce continuous data. "This will provide 100,000 times more information, with much better resolution throughout the sun," says John Leibacher at the National Solar Observatory in Tucson, Arizona, lead scientist for the project. Some of the initial findings will be published in *Science* next spring. In the meantime, the GONG World Wide Web site at **http://helios.tuc.noao.edu** will carry updates and images.



ODP

Survivor. *JOIDES Resolution.*

Rough Seas For Ocean Drilling

"When you're out at the edge of the envelope trying to do science, sometimes you get bitten," observes Jeffrey Fox, director of the international Ocean Drilling Program (ODP). And sometimes you get beaten. Fox should know: Early this month a huge storm off Greenland battered ODP's 143-meter ship, the only deep-sea scientific drilling vessel in the world, crippling its navigation, accessory propulsion, and communication systems. The ship limped into port, but researchers have lost time, money, and perhaps some future drilling opportunities.

ODP, at $45 million per year the largest ongoing earth sciences program, has been venturing into higher, more dangerous latitudes since the *JOIDES Resolution* replaced the smaller *Glomar Challenger* in 1985. Off southeast Greenland a few weeks ago, 50 shipboard scientists were going after volcanic crustal rocks spewed when Greenland and Europe ripped apart 60 million years ago.

Those plans changed abruptly when a storm predicted to be of modest intensity whipped itself into hurricane-force winds with gusts up to 190 kilometers per hour. Two 30-meter waves smashed a window and flooded the bridge, damaging the ship's communication and navigation systems. Two of the 70-ton thrusters that keep the ship positioned over a drill hole also took hard hits. "Everyone feels lucky to be alive after those 26 hours," says marine geologist Robert Duncan of Oregon State University, a co-chief scientist on the cruise.

The ship struggled into Halifax, Nova Scotia, where it is now undergoing repairs. Meanwhile, the program is tallying its losses: a repair bill of perhaps several hundred thousand dollars—although insurance will pay some costs—plus almost a month of lost days at sea that run $60,000 a day.

Fox says he's hopeful the ship will be ready for the next cruise by 1 November. ODP will now be considering just how late to work into the summer drilling season, he says. But he insists "we won't be shrinking from drilling in high latitudes."

Tech Help for Schools

While high-tech education visionaries are issuing apocalyptic warnings about the fate of the allegedly backward U.S. school system, a privately sponsored group has launched a Peace Corps–style initiative to ease schools into the 21st century.

The Massachusetts-based Tech Corps, which recruits volunteers to help schools harness computers and telecommunications technology, announced last month that it is going national. The corps, whose chief sponsor is the Cellular Telecommunications Industry Association, will hold a conference in Washington, D.C., on 30 October where people can learn how to start state chapters. Founded by Gary J. Beach, chief executive officer of Computerworld Inc., the corps last year recruited 300 volunteers through the magazine *Computerworld* who have been working in 12 school districts throughout Massachusetts.

Karen Smith, Tech Corps' national director, says the response to the call for volunteers, from people in industry, government agencies, and private consulting firms, has been "incredible." They help school districts in any way they are asked—such as by installing wiring, training teachers, persuading local industry to donate hardware, and helping schools to construct World Wide Web home pages.

Interested parties can reach Tech Corps' own Web pages at **http://www.ustc.org/**.

Freshwater Ecosystems and Their Management: A National Initiative

Robert J. Naiman, John J. Magnuson,
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Fresh water is a strategic resource that structures the nation's natural and cultural landscapes and is a major determinant of regional economies and demographic patterns. Water consumption in the United States has more than doubled since 1940 and is likely to double again within the next 20 years (*1*–*3*). Critical water-related challenges now face the nation regarding availability, human health and safety, and environmental integrity. These challenges persist despite numerous federal laws (such as the Clean Water Act, Safe Drinking Water Act, Endangered Species Act, Forest Practices Act, and National Environmental Policy Act) and state provisions regarding surface water, ground water, and water rights.

Collectively, these laws and their implementing regulations have created a legislative and judicial pastiche that does not allow the integration necessary to resolve issues related to fresh water. The Clean Water Act, for example, has been the foundation for water quality programs nationwide since 1972. In many ways it has been successful: Sewage treatment and drinking water supplies have improved, and severely polluted systems such as the Cuyahoga River and Lake Erie show signs of renewed vitality. But the Clean Water Act's focus on point-source pollution shifted emphasis away from other equally harmful and pervasive forms of environmental degradation, such as altered hydrological regimes, habitat destruction, invasions by exotic species, and nonpoint-source pollution (*4*, *5*). In addition, the Clean Water Act failed to provide a framework for identifying research priorities, making decisions, or directing broader statutory attention. Similar problems exist with other legislation; each law does some good but also shifts attention away from competing or broader issues. No one law provides a comprehensive approach.

These concerns are exemplified in current discussions about reauthorization of the Clean Water Act and the Endangered Species Act. The nation would benefit if these discussions were centered on how to improve protection and restoration of water resources and aquatic species, and how to integrate human needs with protection and rehabilitation. Instead, the discussions are more ideological than factual. The Clean Water Act Amendments of 1995 (H.R. 961) reverse many of the advances made since 1972 by providing pollution waivers to industries, decreasing wetland protection and sewage treatment, loosening rules against contaminated runoff, and compensating landowners not to harm public resources. If we are to develop a workable plan for fresh waters, decisions must be based on an understanding of freshwater ecosystems and on more effective and comprehensive laws and policies.

Scientists, managers, and politicians are routinely called on to address competing demands on freshwater supplies and ecosystems, but they are increasingly unable to respond at scales commensurate with the issues. Why? Policy development and management activities are frequently undertaken without an adequate empirical foundation; inappropriately short-term, single-focus approaches are accepted with little question; human-caused change is often difficult to distinguish from natural variation; and even when relevant data are available to guide decision making, the legal and regulatory framework is inadequate (*6*, *7*). Consequently, the criteria for effective management and policy decisions are ambiguous at a time when degradation of water supplies and aquatic resources is accelerating.

Meeting human needs for the goods and services provided by freshwater systems can be accomplished only if the people of the United States improve the local, state, and federal institutions charged with understanding, protecting, and managing fresh waters. Collectively, the institutions must be able to predict the consequences of human actions on the aquatic resources, provide an integrated socioenvironmental perspective, and respond to present and emerging issues through education and research.

Research Priorities

Given the complexity of the challenge and the inherent institutional constraints, scientists and managers recently identified six freshwater priorities on the basis of scientific significance, sociopolitical relevance, and the needs of decision makers (*8*).

Ecological restoration and rehabilitation. To recognize human-induced degradation and to guide effective restoration and rehabilitation, we must understand how natural systems—from molecular to watershed scales—operate. Even though many rehabilitation activities are under way, in most cases the approaches do not allow for learning by adaptive management (*9*). Restoration and rehabilitation are a high priority because, whatever the advances generated by the Clean Water Act and related legislation have been, water quality does not meet current standards in one-third of the nation's freshwater ecosystems. The actual proportion is closer to two-thirds if more comprehensive biological criteria are used. The reported proportions of substandard water quality also vary among freshwater environments assessed: one-third for rivers, >50% for lakes, 98% for Great Lakes shorelines, and 44% for estuaries (*5*, *10*). In addition, commercial fish harvests have declined drastically, fish consumption advisories occur in at least 45 states each year, and many aquatic species are threatened with extinction (*4*–*6*, *8*). Most of the nation's freshwater systems are best characterized as ecologically impoverished (*5*, *10*).

Maintaining biodiversity. The goal of maintaining biodiversity includes not only individual species but also the diversity of ecological processes and the integrity of ecological systems. Understanding relations between species and ecological processes as well as the consequences of exotic invasions (for example, the zebra mussel and the stocking of game fish) is fundamental. The nation's capacity to address this area is rapidly declining: Fewer systematists are being trained, and opportunities for applying molecular techniques to species identification remain limited. In the United States, the proportion of freshwater biota classed as rare or in danger of extinction ranges from 34% for fish to 65% for crayfishes and 75% for unionid mussels. Of 214 stocks of Pacific salmon, 74% face a high or moderate risk of extinction. Despite massive expenditures to improve water quality, none of the 251 fishes listed as rare in 1979 was removed from the list in 1989 because of successful recovery efforts (*11*). Less conspicuous species languish in obscurity; for example, only about 30% of commonly collected immature forms of aquatic insects are readily identifiable (*8*).

Modified hydrological flow patterns. With the exception of Alaska, the hydrological regime in virtually every body of fresh water in the United States has been modified to some extent by dams, diversions, and with-

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drawals (2, 12). Hydrological changes have greatly modified conditions for riparian and aquatic organisms in major ways: Habitats for organisms adapted to natural discharge and water level patterns are reduced, rivers are much less able to serve as migratory and material transport corridors, and riparian zones no longer serve as filters between upland and aquatic systems (2, 12).

Ecosystem goods and services. Modifications have severely altered the resources provided by freshwater ecosystems: water quantity and quality, biological productivity and other living functions, and aesthetics and recreation. Improved understanding of the environmental factors responsible for these benefits and their values—including the costs associated with their loss—is necessary for responsible management.

Predictive management. Three types of uncertainty—"noise," unknown but potentially knowable states of nature, and surprises (13)—frustrate ecologists and managers because they perpetuate resource management failures. Data are needed on disturbance regimes and their physical and biological legacies if we are to predict the consequences of cumulative and synergistic impacts.

Solving future problems. Many pressing issues associated with fresh waters are unsolvable at present, yet they promise to become increasingly complex, contentious, and strategic for the United States as demographic patterns, resource consumption, environmental quality, social and institutional organization, and technologies change. Large interdisciplinary programs as well as single investigator–initiated basic research have proven their worth as investments in detecting and solving unforeseen problems (6, 8). We must ensure that basic science and education can provide the framework for meeting emerging water-related challenges.

Achieving Coordination

Research priorities can only be useful if they are effectively coupled to management and policy. How can that complex link be forged? An effective national water policy requires the coordination of the individuals and institutions that plan research and management programs; this could be accomplished through either a strengthened National Biological Service or a central science office. Coordination on a national scale, however, should be integrated with regional needs. The regional level is the primary level for effective management and policy decisions because diverse institutional and political cooperation requires spatial scales that are both ecologically realistic and relevant to human communities.

We estimate that such an approach would cost about $200 million per year, less than 1% of what the United States spends annually on procurement, regulation, and remedial protection of fresh waters. Monies far in excess of what is needed for a comprehensive freshwater program are already being spent on ineffective and contradictory programs (8, 14). For example, according to the Northwest Power Planning Council (Portland, Oregon), more than $150 million is spent annually on the recovery of the degraded salmon and steelhead runs in the Columbia River, yet a monitoring program that would enable the measurement of the major sources of mortality at key points in the river and ocean ecosystem does not exist. With little or no formal peer review, this spending constitutes well over twice the annual budget of the Environmental Biology Program at the National Science Foundation (NSF), which is the primary source of competitive funding for basic research in freshwater ecology in the United States. We do not mean to be critical of any particular program without a full analysis of the situation. Our point is that freshwater research in this nation should be prioritized in relation to documented problems, and that cost effectiveness should be emphasized through scientifically based monitoring and evaluation of all water-related research and management programs.

We envision two broad implementation categories, one focusing on institutions and the other on knowledge (8). The first category includes (i) efforts to strengthen existing agencies to promote understanding, protection, and regulation of freshwater ecosystems and resources; (ii) enhancement of existing agency programs to support innovative research and technology development and transfer; (iii) establishment of regional institutions designed to integrate human and natural sciences and to bring together managers and scientists from government, academia, and the private sector; and (iv) a new, integrated NSF program to promote effective interdisciplinary freshwater research on a scale commensurate with contemporary issues.

The second category addresses professional and public literacy about freshwater ecosystems and their management. It includes (i) a national center to provide data on freshwater biodiversity, develop sensitive biotic indices of environmental change and ecological integrity, and enhance the accuracy and precision of monitoring programs; (ii) an array of long-term natural and human-altered research sites with a specific focus on fresh waters (15); and (iii) the strengthening of education and communication to provide truly innovative training for students and professionals in freshwater disciplines, including continuing education for midcareer scientists and managers.

Science and policy based on factual information must form the basis for the regulation and rehabilitation of the nation's fresh waters. The nation cannot otherwise protect its long-term cultural, economic, and environmental interests that are intimately tied to fresh water. As we enter the 21st century, demands on water resources will intensify and the need for sound information, an ecologically literate population, and comprehensive legislation will become even more obvious. To protect our freshwater ecosystems, we need knowledge, wise leadership, and real cooperation to find the correct mix of laws, incentives, and regulations as well as the political will to enact them.

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14. Examples of expenditures that consume fiscal resources but do not effectively protect water resources include a nearly exclusive reliance on chemical criteria to meet the goals of the Clean Water Act, the use of hatcheries to maintain fish populations, bounty programs to maintain dwindling fish stocks affected by water resource development, and massive engineering projects that fail to account for the natural dynamics of the freshwater ecosystem.
15. The United States boasts an array of biological field stations, but they generally receive substandard support and are seldom organized to foster long-term integrative research. Likewise, of the NSF's 18 Long-Term Ecological Research sites, only two have a primary freshwater focus.

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Reverse Weathering, Clay Mineral Formation, and Oceanic Element Cycles

Fred T. Mackenzie and Lee R. Kump

Reverse weathering as a sink of oceanic elements was proposed 30 years ago (*1*). According to this hypothesis, the formation of clay minerals in marine sediments leads to the removal of soluble cations and the release of acidity (CO_2), the opposite of the weathering reaction. Conclusive tests of this hypothesis have been difficult to make because the required quantity of authigenic (newly formed) minerals is small and thus extremely difficult to detect in deltaic sediments dominated by clays derived from continental weathering. On page 614 of this issue, Michalopoulos and Aller (*2*) demonstrate the rapid formation of aluminosilicate ("clay") minerals in marine sediments of the Amazon delta using an elegant approach. Rather than attempting to detect naturally occurring products of reverse weathering, the investigators formed their own. They affixed seed materials (including quartz grains, glass beads, and quartz grains coated with iron oxide) onto acrylic slides and inserted then into jars of Amazon sediment. Finding that clay minerals readily form, they go on to calculate that clay mineral formation processes throughout Amazon shelf sediments could consume about 10% of the present global riverine K^+ flux. The muddy deltas of tropical river systems receive about 60% of the continental river particulate flux to the oceans. If these systems have mineral formation characteristics similar to that of the Amazon, then authigenic clay mineral reactions have important implications for problems concerning the global geochemical balance of several major and minor elements.

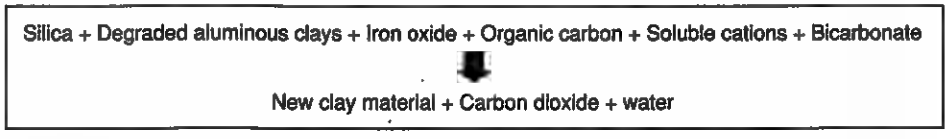
The dissolved constituents that enter the ocean by way of rivers, ground-water flow, and hydrothermal reaction at oceanic ridge sites eventually must precipitate from the oceans or be recycled back to the continents through the atmosphere. In 1966, Mackenzie and Garrels (*1*), inspired by Sillen's paper (*3*) on the physical chemistry of seawater, calculated a mass balance for river water in which the major dissolved constituents of rivers were precipitated from the oceans as common mineral phases found in marine sediments. So that all of the constituents were balanced, part of the Na, Mg, K, and SiO_2 had to be removed by reaction with solid "degraded" aluminosilicate minerals (clays) of the suspended load of streams to make authigenic clay minerals typical of those found in muddy marine sediments. Furthermore, Mackenzie and Garrels pointed out that if the CO_2 consumed in the weathering processes producing dissolved constituents and degraded clays for riverine transport to the oceans is not returned to the atmosphere by depositional processes, there will be a decrease in the atmospheric concentration of CO_2. Seawater, because of the accumulation of bicarbonate ion from chemical weathering, would become alkaline. Any net drain on atmospheric CO_2 would arise because of failure of reversal of the reactions of silicate weathering. The depositional reactions were termed reverse weathering.

We now know that the process envisioned by Mackenzie and Garrels is far more complex. Since their papers, discovery of large-scale hydrothermal cycling of elements at mid-ocean ridges (*4*) has displaced the site of reverse weathering to these environments and, for many scientists, made sedimentary sinks for certain dissolved constituents in the oceans unnecessary. This is despite the fact that problems concerning the river-ocean mass balance still exist and can be resolved if early diagenetic formation of clays is a viable process (*5*).

Michalopoulos and Aller's paper has reopened the Pandora's box of reverse weathering in sediments (*2*). These authors observed the formation of significant quantities of K-Fe-Mg clay minerals precipitated on naturally occurring substrates incubated in unaltered, anoxic, Amazon delta sediments for 12 to 18 months at 28°C. Phases formed both between substrate mineral grains as direct precipitates from solution (cements) and by alteration of FeOOH-rich coatings on substrate grains (a process of reconstitution). Painstaking scanning and transmission electron, energy-dispersive system, x-ray powder diffraction, and wave-dispersive electron microprobe analyses support the authors' conclusion that the dominant phase formed was a K-Fe-rich clay of 9.9 Å, a mica-type clay mineral. The incubation experiments demonstrate the potential for the rapid formation of clay minerals in Amazon delta muddy sediments and their likely structure and average composition. A variety of field evidence (*6*) suggests that clay minerals consistent with the composition of the experimental precipitates do indeed form in Amazon delta sediments. The formation or reconstitution of clay minerals in these sediments is responsible for the uptake of about 7% of the global riverine supply of fluorine to the ocean and about 10% of the potassium input.

The formation of clay mineral precipitates and reconstitution of FeOOH coatings in Amazon delta sediments require a source of Al and Si. The Al source may be the dissolution of relatively unstable aluminous phases typical of tropical weathering regimes or may come from the dissolution of biogenic, diatom-derived silica that has scavenged Al and been transported to the sediment. Whatever the case, Al does not act as an immobile component in Amazon delta muddy sediments and takes part in rapid dissolution-precipitation reactions involving formation of authigenic clay minerals. This finding runs counter to current scientific dogma claiming that Al is an immobile component in low-temperature environments. It appears that the major limitation on the amounts of clay minerals formed in Amazon delta sediments is the supply of reactive silica derived from diatom dissolution (perhaps also from degraded aluminosilicates). If silica does control the amount of newly formed clay mineral, then authigenic clay formation becomes a sink for biogenic silica in the ocean, as originally suggested by Mackenzie and Garrels (*1*).

The net reaction scheme proposed by Michalopoulos and Aller for the formation of new clay mineral phases in Amazon delta sediment (see figure) is remarkably similar



New clays for old. Generalized schematic reverse weathering reaction leading to the formation of clay minerals in Amazon delta muddy sediments. Notice that this reaction leads to the removal of soluble cations and the release of acidity (CO_2). This reaction is the opposite of the chemical weathering process.

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to that proposed for the formation of authigenic clay minerals (nontronite and illite-smectite) in marine muddy sediments of Kaneohe Bay, Hawaii (*7*) derived from tropical weathering of basalts. This implies that reverse weathering reactions similar to those proposed by Michalopoulos and Aller may be more characteristic of nearshore muddy sediments than indicated by studies in the early 1970s of sediments in temperate environments (*8*). If so, the large quantities of degraded tropical weathering products entering nearshore environments portend substantial formation of authigenic clay minerals. Mackenzie and Garrels (*1*) pointed out that only 7% of the mass of sediments accumulating on the sea floor needs to undergo diagensis (formation of new clay phases or reconstitution of detrital phases) to resolve the mass balance between rivers and oceans. However, before reverse weathering reactions can be viewed as a significant component of elemental cycling in the ocean, it must be demonstrated that these reactions have a global significance. To do so requires more studies of the kind carried out by Michalopoulos and Aller, particularly in muddy nearshore environments of the tropical environment.

What happens to the large quantities of degraded aluminosilicates transported to these environments by tropical rivers? They are not found in shallowly buried sediments, indicating their potential for taking part in early and rapid diagenetic reactions. It is very likely that the ultimate resolution of the geochemical balance for the ocean will involve hydrothermal and low-temperature alteration of the basaltic oceanic crust and sedimentary sinks for the major and minor elements, including the incorporation of elements in newly formed or reconstituted clay mineral phases.

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Metal-Carbon Bonds in Nature

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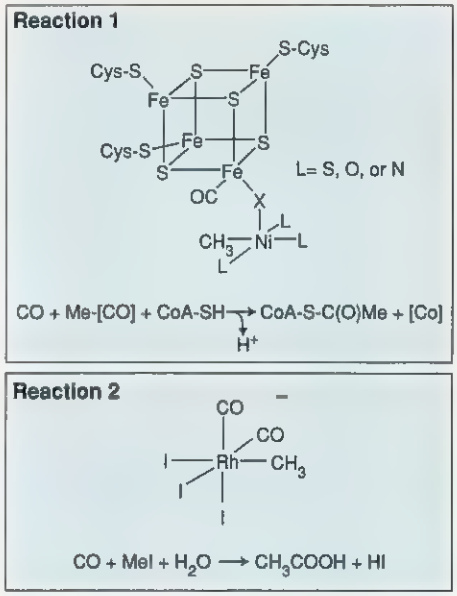
Synthetic organotransition-metal catalysts (species possessing a direct metal-carbon or metal-hydrogen bond) are frequently used in industrial processes to convert hydrocarbon fragments into industrially useful chemicals (*1*). Transition-metal alkyl ($M–CR_3$) species may, in many instances, play an important role as intermediates in these reactions. In contrast, biology tends to utilize CO_2 or CO to form metabolically useful compounds. There is, however, one biological system, which is fondly referred to as "nature's organometallic catalyst," namely vitamin B_{12} (*2*), that makes use of a $M–CR_3$ species. Vitamin B_{12} contains cobalt in a substituted corrin macrocycle (a flexible porphyrin relative) and contains an axial Co(III)–alkyl (CR_3). The macrocyclic environment imparts special properties to the cobalt center that allow it to function as nature's Grignard reagent (CR_3^- source), radical ($CR_3\bullet$ source), or Meerwein's reagent (CR_3^+ source). The reaction-type promoted by this site depends on the mechanism of Co–C bond cleavage. The accessibility of several different oxidation states (+1, +2, +3) allows the versatile behavior of this site. On page 628 of this issue, a report by Kumar *et al.* (*3*) presents strong evidence for the occurrence of a second organometallic intermediate in biology that consists of a reactive $Ni–CH_3$ fragment, which serves as a precursor to acetic acid through its reaction with CO and CoASH (acetyl-CoA synthase reaction 1). Unlike vitamin B_{12}, the nickel ion in this enzyme, carbon monoxide dehydrogenase (CODH), is coupled to an iron-sulfur Fe_4S_4 cluster (Fig. 1).

Iron sulfur clusters are ubiquitous in nature, and analogs are accessible by means of synthetic methods (*4*, *5*). Up until the early 1980s, these clusters were thought to function solely as electron transfer and storage sites, delivering electrons to enzymes that promote substrate reduction (the most difficult being N_2 reduction to ammonia). Later, it was shown that clusters of this type can serve as enzyme active sites (such as Aconitase) and bind and activate substrate (*6*). Examples of Fe_4S_4 clusters linked to a more reactive substrate binding site M (*7*), by what is referred to as a bridging ligand X ($M–X–Fe_4S_4$), are found in an increasing number of biological systems [for instance, M= Fe(siroheme) in sulfite reductase] (*8*, *9*). If the two sites are chemically linked, they can communicate so that substrate binding at the M site triggers facile multielectron transfer, thereby avoiding toxic or unstable intermediates. In some cases the M site is incorporated into the cubane core (MFe_3S_4) in place of one of the irons (*10*).

With CODH (*4*, *11*) the properties (such as Mössbauer spectra) of the Fe_4S_4 core are not dramatically affected by the presence of the M = Ni site, suggesting that the nickel is bound externally to the cluster core. This led Lindahl, Ragsdale, and Münck to propose the cluster core structure shown in the figure (*12*). Synthetic modeling studies appear to support this structure (*13*). The identity of the bridging ligand X is unknown. Evidence for coupling between the Ni site and the Fe_4S_4 cluster derives from studies in which isotopic labels (^{61}Ni, ^{57}Fe, ^{13}CO) incorporated into the CO derivative perturbed the electron paramagnetic resonance (EPR) signal associated with this cluster site (*14*). Resonance Raman and infrared studies established that CO binds to one of the iron sites (*15*, *16*). Reaction of the CO-bound derivative with a methylated corrinoid (Me-[Co]) species results in methyl transfer to the nickel site with the formation of a CH_3-bound intermediate (see figure). Evidence



Proposed intermediates for the biological acetyl-CoA synthase reaction (reaction 1) and the Monsanto acetic acid process (reaction 2).

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for CH_3 binding to the Ni site is presented in this issue of *Science* (*3*).

To complete the biological reaction, CoASH reacts with the intermediate shown in the figure to form the thioester CoAS-C(O)Me (reaction 1). The mechanism by which this C–C bond-forming process occurs is still under scrutiny (*16*, *17*). The simplest explanation would involve the formation of an intermediate acyl [M-$C(O)CH_3$] (M = Fe or Ni) species, and evidence does indeed point to an acyl intermediate in reaction 1. It is not clear whether an Fe^- or Ni^- acyl intermediate is involved. With synthetic organometallic systems, CO insertion into M–CH_3 bonds, to form M–$C(O)CH_3$, is one of the most fundamental reaction types (*1*). These reactions generally occur at mononuclear (single metal) sites that contain electron-accepting supporting ligands such as PR_3, C_5H_5 (Cp), or CO. Acyl formation occurs by means of methyl migration to the carbon of an adjacent CO. The best known example of this is the Monsanto acetic acid process (reaction 2), the catalyst of which is shown in the figure. An obvious distinction between the biological system CODH and synthetic catalysts is the absence of electron-accepting PR_3, Cp, or CO ligands, because they are not biologically available. Instead, nature is limited to ligands L-S, N, and O, which are not typically found to encourage acetate formation. A few rare examples of synthetic models for the CODH Ni site containing biologically relevant ligands (S, N, or O) have been reported, however (*18*–*21*). Mononuclear (S, N)-ligated Ni–CH_3 species have also been shown (*17*, *18*, *21*) to react with CO to form acyl complexes, and then to convert to thioesters upon addition of thiols. This is directly relevant to the proposed pathway of acetyl-CoA synthase reactivity. Complexes of Ni(I)–CO with biologically relevant ligands are also known (*18*, *20*).

The question that remains concerns the role of the Fe_4S_4 cluster in CODH. Given the synthetic model reactions described above, it would appear that Ni is capable of undertaking the entire CODH reaction scheme without the aid of an Fe_4S_4 cluster. In fact, synthetic Fe_4S_4 clusters are unstable in the presence of CO under reducing conditions (*22*). It has been proposed (*16*, *17*) that the more oxidized Fe_4S_4 cluster serves as a CO binding site, and that CO insertion, involving the Fe_4S_4–CO intermediate shown in the figure, is promoted by redox changes at the cluster site. This has yet to be synthetically modeled. Synthetic models have shown, however, that in order for thioester formation to take place at a Ni center, the Ni ion must be reduced by $2e^-$ [from Ni(II) to Ni(0)] (*17*). It is therefore possible that the Fe_4S_4 cluster in CODH serves to facilitate the removal of these two electrons, a step that appears to be critical to the stability of the Ni site. The reconciliation of the mononuclear pathway of acyl formation observed with synthetic systems, and the binuclear pathway proposed to occur with CODH (*16*, *17*), awaits further study.

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Calcium Sparks in Vascular Smooth Muscle: Relaxation Regulators

Fredric S. Fay

Many smooth muscle cells periodically exhibit spontaneous transient outward (hyperpolarizing) currents, or STOCs (*1*). Each of these events results from the opening of 10 to 100 potassium-selective channels, triggered simultaneously by a rise in cytoplasmic calcium concentration ($[Ca^{2+}]$). Because these STOCs can be suppressed by agents that interfere with the release of Ca^{2+} from intracellular stores, the triggering Ca^{2+} for the STOCs has been presumed to come from inside the cell (*1*, *2*). It has so far been impossible to detect the rise in Ca^{2+} that activates the STOCs—presumably because the Ca^{2+} increase is highly localized and brief, and thus invisible in whole-cell Ca^{2+} recordings or Ca^{2+} images with low time resolution. Now, Nelson and co-workers report in this issue of *Science* the first sighting of these local increases in Ca^{2+} in the cytoplasm of single smooth muscle cells (*3*). This first glimpse of these "Ca^{2+} sparks" is exciting for understanding how STOCs are generated, but perhaps even more exciting is the demonstration that sparks are quite likely responsible for a specific cell function—a vasodilatory influence on small cerebral arteries.

The Ca^{2+} sparks of smooth muscle are not quite the same as those in cardiac muscle, the tissue in which sparks were first reported (*4*). The Ca^{2+} sparks in both muscle types do have a similar duration (~100 ms), magnitude (a few hundred nM), and spatial extent (2 μm diameter at half-maximal $[Ca^{2+}]$). In both tissues, the Ca^{2+} sparks arise from the opening of one or several ryanodine receptors and reflect the activation of an elementary Ca^{2+}-release unit. In cardiac muscle, the sparks are recruited throughout the cell to produce the global rise in $[Ca^{2+}]$ that causes the synchronous activation of the contractile system and the consequent ejection of blood from the heart (*5*). However, the Ca^{2+} sparks in smooth muscle are generated in isolation principally near the cell surface, presumably reflecting the fact that in smooth muscle the sarcoplasmic reticulum (SR), enriched in ryanodine receptors, is near the cell surface (*6*). These ryanodine-sensitive release units are thus perfectly positioned to receive signals from the plasma membrane and to send signals in the form of localized Ca^{2+} increases. In cardiac muscle, the ryanodine receptor amplifies Ca^{2+} signals arising from the plasma membrane. The studies of Nelson and co-workers, however, show

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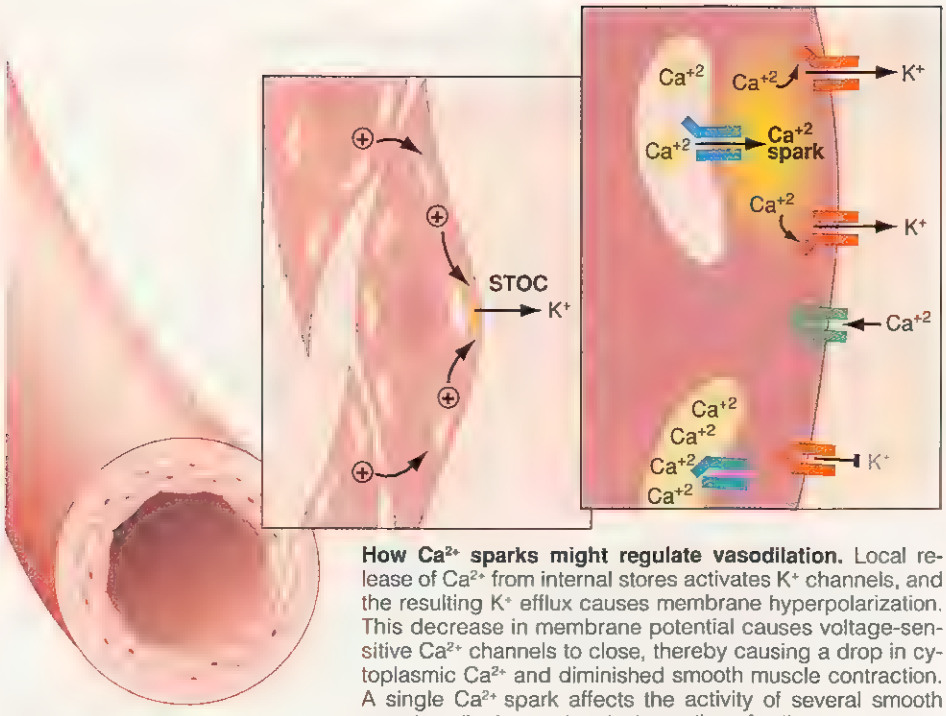

ILLUSTRATION K. SUTLIFF

How Ca^{2+} sparks might regulate vasodilation. Local release of Ca^{2+} from internal stores activates K^+ channels, and the resulting K^+ efflux causes membrane hyperpolarization. This decrease in membrane potential causes voltage-sensitive Ca^{2+} channels to close, thereby causing a drop in cytoplasmic Ca^{2+} and diminished smooth muscle contraction. A single Ca^{2+} spark affects the activity of several smooth muscle cells due to electrical coupling of cells.

that the job of the ryanodine receptor is different in smooth muscle, where receptors on internal Ca^{2+} stores "instruct" channels on the plasma membrane what to do.

Nelson *et al.* present three lines of evidence linking Ca^{2+} sparks and STOCs. (i) The average time course of both events is very similar; (ii) both events originate on or near the surface of the cell; and (iii) agents that interfere with Ca^{2+} sequestration by internal stores inhibit, at similar concentrations, the appearance of both Ca^{2+} sparks and STOCs. Future studies will need to demonstate that the appearance of Ca^{2+} sparks in the same single cell is almost always associated with the appearance of a STOC. (In the report by Nelson *et al.*, STOCs and Ca^{2+} sparks were recorded in different cells under different conditions.) Nelson *et al.* calculate that in smooth muscle cells, about 13 Ca^{2+}-activated K^+ channels are open at the peak of a STOC and suggest that this group of channels is activated by a single Ca^{2+} spark with an average peak value of 300 nM and an area of approximately 3 μm^2.

The Ca^{2+} sparks and the resulting STOCs appear to determine the degree of constriction of small arteries, and hence blood pressure. Agents such as ryanodine and thapsigarin, which interfere with Ca^{2+} sequestration by internal stores, cause membrane depolarization and arterial constriction. Iberiotoxin, which selectively blocks Ca^{2+}-activated K^+ channels, also causes membrane depolarization and vasoconstriction. And in a vessel treated with doses of ryanodine or thapsigarin, which prevent Ca^{2+} sparks and STOCs, iberiotoxin has no further effect. The implication is that under normal conditions, Ca^{2+} sparks that activate Ca^{2+}-sensitive K^+ channels in smooth muscle cells occur throughout the vessel wall. The local Ca^{2+} sparks and more widely spread hyperpolarizing currents exert a tonic hyperpolarizing and inhibitory influence that counters excitatory inputs to the smooth muscle cells.

We do not know what causes the Ca^{2+} sparks in these smooth muscle cells. Work on other systems has taught that opening of the ryanodine receptor channels is a bell-shaped function of $[Ca^{2+}]$ in the cytoplasm (*7*) and that increases in $[Ca^{2+}]$ inside internal Ca^{2+} stores enhance the probability of receptor opening (*8*). Because several agents that affect STOC frequency also affect various protein kinases (*1*), Ca^{2+} sparks may be triggered by local protein kinase activity. Various protein kinases affect ryanodine receptor function (*9*, *10*) and Ca^{2+} pumps on internal stores (*10*). It remains to be determined whether any of these factors triggers the Ca^{2+} sparks of smooth cells and, on the practical side, whether natural and synthetic vasodilators and vasoconstrictors act directly on the Ca^{2+} spark–STOC pathway.

The Ca^{2+}-activated K^+ channels function as endogenous Ca^{2+} detectors. Near the typical resting potential of smooth muscle (-40 to -50 mV), the value at which the experiments of Nelson *et al.* were carried out, a $[Ca^{2+}]$ of at least several micromolar is required for significant activation of these channels (*11*). The peak $[Ca^{2+}]$ detected during a Ca^{2+} spark was only about 300 nM. It is likely, therefore, that the Ca^{2+} sparks are much more localized but larger in amplitude than is revealed by these imaging methods. As new submembrane-localized Ca^{2+} indicators (*12*) and higher resolution imaging methods (*6*) are applied, these Ca^{2+} sparks will likely appear brighter and smaller. The average density of Ca^{2+}-activated K^+ channels on the smooth muscle cell membrane is estimated to be about 1 to 2 channels per μm^2 (*13*). Hence, to accommodate the coupling of a single Ca^{2+} spark—which causes the $[Ca^{2+}]$ to rise sufficiently to activate these K^+ channels in a region less than 1 μm^2— requires that these Ca^{2+}-activated K^+ channels are not uniformly distributed on the cell surface but instead are clustered in regions closely apposed to and in register with elements of the SR. Clustering of ion channels, exchangers, and pumps on the plasma membrane of smooth muscle has been recently observed (*14*) in close proximity to regions of the SR enriched in ryanodine receptors (*6*). Because Ca^{2+} is believed to diffuse very slowly through the cytoplasm (*15*), close approximation of Ca^{2+}-release sites to Ca^{2+}-sensitive targets is required, especially for the transmission of brief and small Ca^{2+}-release signals. Hence, the position of ryanodine receptors on internal stores relative to plasma membrane Ca^{2+}-activated K^+ channels and voltage-gated Ca^{2+} channels may well determine whether in a given cell ryanodine receptors amplify incoming Ca^{2+} currents or stimulate hyperpolarizing current as reported by Nelson and co-workers in cerebral arterial smooth muscle cells.

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Mechanisms for Lithium Insertion in Carbonaceous Materials

J. R. Dahn,* Tao Zheng, Yinghu Liu, J. S. Xue

Lithium can be inserted reversibly within most carbonaceous materials. The physical mechanism for this insertion depends on the carbon type. Lithium intercalates in layered carbons such as graphite, and it adsorbs on the surfaces of single carbon layers in nongraphitizable hard carbons. Lithium also appears to reversibly bind near hydrogen atoms in carbonaceous materials containing substantial hydrogen, which are made by heating organic precursors to temperatures near 700°C. Each of these three classes of materials appears suitable for use in advanced lithium batteries.

There are hundreds of commercially available carbon types, including natural and synthetic graphites, carbon blacks, active carbons, carbon fibers, cokes, and a wide variety of other materials prepared by the pyrolysis of organic precursors in inert gas. All of these materials reversibly react with lithium to some extent and can be used as the negative electrode in lithium-ion batteries (*1*). To maximize the stored energy per unit mass in these batteries, it is crucial to determine which types of carbon react reversibly with the largest amount of lithium per unit mass of carbon. Hundreds of carbon types have been tested for this application (*2*) and three classes of commercially relevant lithium-battery carbons have emerged.

Carbon Types

Figure 1 shows the maximum amount of lithium with which carbons can reversibly react as a function of the heat treatment temperature for carbons prepared by the pyrolysis of organic precursors. These data were collected by electrochemically reacting lithium with the carbons in cells with lithium metal as one electrode and with a particular carbon sample as the other electrode. In such an experiment, the Li atoms are transferred from the metal to the carbon as ions (through the cell electrolyte) and electrons (through the external circuit). Battery scientists measure the number of electrons transferred through the external circuit per gram of carbon; this quantity is the specific capacity of the carbon in milliampere-hours per gram. Because one lithium ion is transferred for each electron, the specific capacity can be related to the stoichiometry of the carbon electrode. For example, if one Li atom is transferred per six C atoms [the maximum limit for graphite under ambient conditions (*2*)], a specific capacity of 372 mA·hour g^{-1} results.

Most striking about Fig. 1 is the large variation in specific capacity that occurs as a function of heat treatment temperature and carbon type. To understand this variation we must consider the structure and chemistry of carbons prepared by the heating of organic precursors. Fortunately, this is a well-studied field (*3*). During the early stages of pyrolysis in inert gas (below 600°C), organic compounds decompose and emit gases that contain carbon, such as CO and CH_4 (*4*). The remaining C atoms condense into planar aromatic structures (called graphene sheets) that are terminated predominantly with H atoms at their edges. If the decomposing precursor forms a semifluid state, then these planar sheets can align in a more or less parallel fashion that leads ultimately to easy graphitization upon heating to very high temperatures. Such precursors yield "soft" or graphitizable carbons. However, if the organic precursor is sufficiently cross-linked, then a fluid state is not realized during decomposition and the planar aromatic structures cannot align. These materials are difficult to graphitize at any heat treatment temperature and thus are called "hard" or nongraphitizable carbons.

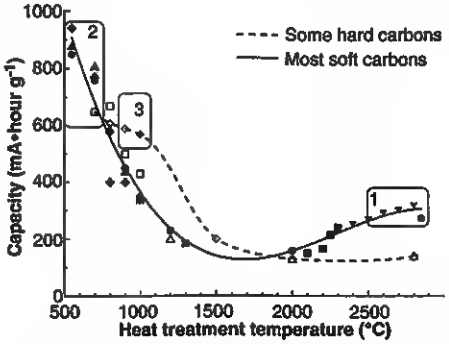

Fig. 1. Plot of reversible capacity for lithium versus heat treatment temperature for a variety of carbon samples (open symbols, hard carbons; solid symbols, soft carbons). These data are for the second charge-discharge cycle of lithium-carbon test cells. The three regions of commercial relevance are shown.

During heating in the temperature range from 600° to ~1000°C, hydrogen is eliminated (*4*) and the size of the planar graphene sheets grows to ~20 to 30 Å. In soft carbons, there are regions where 3 to 10 of these sheets are stacked in a more or less parallel fashion, separated by small regions of "unorganized carbon" that may consist of buckled single layers or tetrahedrally bonded carbon (*5*). The layers are stacked with random rotations or translations between them, a condition known as turbostratic disorder (*6*). In some hard carbons, the graphene sheets do not stack in a parallel way and are in a "house of cards" arrangement. Such carbons inherently have appreciable nanoporosity, with pores on the order of the size of the graphene sheets (*7*).

During further heating of soft carbons above 1000°C, the lateral dimensions of the graphene sheets grow to ~150 Å; by 2000°C, the layers become parallel (with 50 to 100 layers per stack) but turbostratic misalignment is not relieved, apparently because of some "pinning" that prevents the rotation of layers into the normal stacking found in graphite. Only above 2000°C is enough thermal energy present to overcome this pinning and for the layers to rotate into the registered graphite stacking arrangement. The probability P of finding adjacent graphene sheets in turbostratic misalignment decreases from ~1 at 2000°C to near 0 for soft carbons heated to ~3000°C. As hard carbons are heated above 1000°C, the size of the graphene sheets grows and the sheets swing into more or less parallel positions, eliminating the nanoporosity. However, large numbers of parallel layers never form at any heat treatment temperature and turbostratic misalignment is never relieved.

Carbons in the three highlighted regions of Fig. 1 are currently used or have been proposed for use in commercial lithium-ion batteries. Region 1 contains carbons prepared by heating so-called soft carbon precursors to temperatures above ~2400°C, where well-graphitized materials result. In these materials, the probability P for turbostratic misalignment between adjacent layers decreases with heating temperature. Region 2 contains both soft and hard carbon precursors that contain substantial hydrogen. As the temperature of the samples is

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increased, the hydrogen content decreases. Region 3 contains hard carbon materials that are made up predominantly of single carbon sheets that contain appreciable nanoporosity and are stacked more or less like a house of cards. All of the data in Fig. 1 were collected in our laboratory. Carbons in regions 1, 2, and 3 have been discussed in (*8–10*), respectively, where we proposed mechanisms for the variation of specific capacity with heating temperature in each region.

Figure 2 shows the voltage-capacity relations for lithium-carbon electrochemical cells made from representative materials from each of the three regions of Fig. 1. The synthetic graphite sample gives a reversible capacity of ~355 mA·hour g^{-1}. Petroleum pitch heated to 550°C to give $H_{0.4}C$ (*9*) gives a reversible capacity of near 900 mA·hour g^{-1}, one of the largest capacities of any material in region 2. All materials in region 2 are anomalous because their voltage profiles show appreciable hysteresis (lithium inserted near 0.0 V is removed near 1 V). Resole resin heated to 1000°C has a composition of $H_{0.04}C$ and gives a reversible capacity of ~560 mA·hour g^{-1}, one of the largest capacities known for materials in region 3. The voltage profiles for each of the materials in Fig. 2 are markedly different, which suggests that different reaction mechanisms are important in each of the three regions in Fig. 1.

Graphitic Carbons—Region 1

Graphite can intercalate up to one Li atom per six C atoms under ambient conditions (*11*). In LiC_6, the Li atoms occupy next nearest-neighbor sites, separated by 4.25 Å, within the van der Waals space between every pair of carbon sheets (*12*). When the lithium is intercalated, it transfers most of its 2*s* electron density to the carbon host and exists as a screened ion between the carbon sheets. The Coulomb repulsion between lithium ions in nearest-neighbor sites is apparently greater than the binding energy of the lithium to the graphite host, so that mixtures of metallic lithium and Li_xC_6 coexist when samples of composition $x > 1$ in Li_xC_6 are attempted at room temperature and pressure.

Recently, Nalimova *et al.* (*13*) showed that samples of $x > 1$ could be prepared in graphite under the extreme conditions of 280°C and 50 kbar on molten lithium in contact with the graphite. Apparently, these conditions are sufficient to induce the occupation of nearest-neighbor sites in Li_xC_6. However, we do not believe that nearest-neighbor sites are occupied in any of the carbons in regions 1, 2, and 3 under ambient conditions for the following reason: Lithium intercalates into solids because its chemical potential is lowered relative to that in elemental lithium. The change in chemical potential includes a $P\Delta V$ term, which can be estimated. If we assume that the molar volume of lithium metal or liquid is 14 cc mol^{-1} and that 6 mol of graphite expands by 10% upon intercalation of 1 mol of lithium, then $\Delta V \approx -11$ cc per mole of intercalated Li and $P\Delta V/P \approx 10^{-5}$ eV $atom^{-1}$ bar^{-1}. At ambient pressure, this contribution to the chemical potential change is negligible, but at 50 kbar, $P\Delta V \approx -0.5$ eV $atom^{-1}$. Therefore, at 50 kbar, we are effectively able to access voltages down to ~−0.5 V versus Li at ambient pressure. If the insertion of lithium into nearest-neighbor sites requires 50 kbar, then it effectively occurs near −0.5 V in Fig. 2. In the experiments of Fig. 2, such voltages can not be reached, because metallic Li electrodeposits on the carbon surface at all voltages below 0 V in equilibrium. Thus, it is unlikely that near-neighbor sites can be filled by lithium in carbonaceous materials under ambient conditions.

When lithium intercalates between adjacent parallel carbon sheets, these sheets rotate from "AB" registry (*14*) into "AA" registry (*15*), with honeycombs directly above and below one another. The Li atoms are centered between honeycombs above and below; therefore, lithium might not intercalate between adjacent layers that are turbostratically misaligned and pinned. Figure 3 (*8*) is a plot of the reversible capacity of region 1 carbons (heated above 2200°C) versus *P*, the probability of turbostratic misalignment in adjacent parallel layers. The capacity *Q* varies as $Q = 372(1 - P)$, as expected on the basis of the arguments above; this finding suggests that no lithium can be inserted between adjacent parallel layers that are turbostratically misaligned.

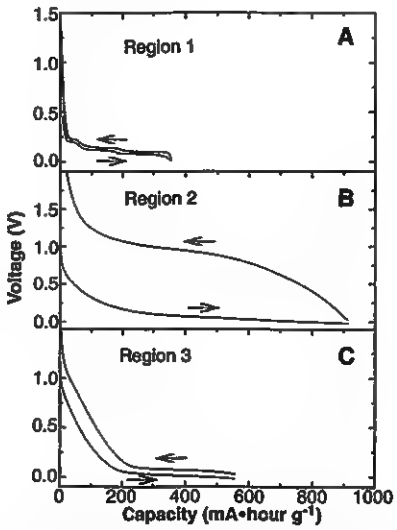

Fig. 2. Plots of voltage versus reversible capacity for the second charge-discharge cycle of representative carbon samples from regions 1, 2, and 3 of Fig. 1. (**A**) Synthetic graphite (Johnson-Matthey); (**B**) petroleum pitch (Crowley Tar Co.) heated to 550°C; (**C**) resole resin (Occidental Chemical Co.) heated to 1000°C. Arrows designate the directions the curves are traversed as Li is added to (to the right) or removed from (to the left) the carbon samples.

Hydrogen-Containing Carbons—Region 2

A variety of materials pyrolyzed at temperatures near 700°C show behavior similar to that in Fig. 2B (*16–18*). Mabuchi *et al.* (*16*) argued that large amounts of lithium are being inserted within "cavities" in these materials. However, our recent work (*10*) shows that these materials offer little evidence for microporosity. Moreover, such an explanation would require the chemical potential of the inserted lithium to be very close to that of metallic lithium, because it would only be weakly bound to the carbon; in experiments like those of Fig. 2B, a cell voltage of several tens of millivolts would be expected, and no hysteresis would be expected. Sato *et al.* (*17*) proposed that the Li atoms intercalate and occupy nearest-neighbor sites between each pair of graphene sheets. This explanation would require some mechanism for the lithium ions to overcome the large screened Coulomb repulsion between ions on nearest-neighbor sites in the absence of a huge applied pressure on the lithium. Sato's mechanism invokes the equilibrium occupation of these sites, so this explanation would not be expected to lead to hysteresis in the voltage profile.

Yata *et al.* (*18*) realized that carbons prepared at these temperatures contain substantial hydrogen and therefore called the materials they prepared from pyrolyzed phenolic resins "polyacenic semiconductors." Typically, the materials that showed high capacity for lithium in (*16–18*) had H/C

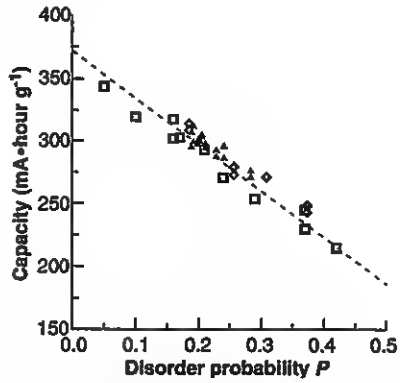

Fig. 3. Reversible capacity of region 1 carbons plotted as a function of the probability *P* of turbostratic disorder between adjacent carbon sheets. The line is the relation $Q = 372(1 - P)$, where *Q* is the capacity. For the purposes of this plot, samples corresponding to different symbols are equivalent.

atomic ratios in the neighborhood of 0.2. Because the hydrogen in these materials could be playing a crucial role in the mechanism of lithium insertion, we synthesized a series of materials at different temperatures from petroleum pitches, polyvinyl chloride, and polyvinylidene fluoride. Figure 4 shows the correlation between reversible specific capacity and H/C atomic ratio for these region 2 materials (9). The solid line in Fig. 4 is that expected if each H atom can bind to a Li atom within the solid and if a hydrogen-free soft carbon heated to near 1000°C has a capacity of 300 mA·hour g^{-1}. Even the data from (*16*) fit nicely in the trend. Note that the data in Fig. 4 are for the second charge-discharge cycle of these cells; these materials do not maintain their large capacities for more than a dozen or so cycles (9).

The substantial work that has been done on ternary graphite-alkali-hydrogen materials may be important to our observations in Fig. 4. In a massive review of the subject (*19*), Enoki *et al.* showed that charge transfer from alkalis to hydrogen in carbons is expected. Therefore, we believe that Li atoms can bind in the vicinity of H atoms in these hydrogen-containing carbons. The inserted lithium could transfer part of its 2*s* electron (in a covalent bond) to a nearby hydrogen, resulting in a corresponding change to the H–C bond. This would cause changes in the relative atomic positions of the C and H atoms; these bonding changes would be activated processes, which can lead to hysteresis. Bonding changes in the host have been shown to cause hysteresis in such electrochemical measurements (*20*). During charging, when the lithium is removed from the carbon, the original H–C bonds would reform. If the reforming of the bonds is not complete, then it is likely that the cycling capacity would slowly decay, as observed (9). We stress that we do not believe that bulk lithium hydride is being formed in these materials, and further work is needed to determine exactly how the lithium is attached near the hydrogen.

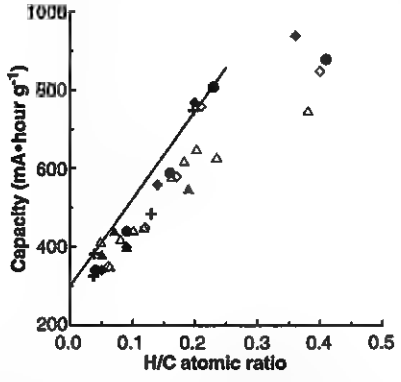

Fig. 4. Reversible capacity of region 2 carbons plotted as a function of their hydrogen content. Samples [●, Crowley pitch; ◇, KS pitch series A (Kureha Co., Japan); △, KS pitch series B (Kureha); ◆, polyvinyl chloride; ▲, polyvinylidene fluoride] were heated to temperatures between 550° and 1000°C. Data from (*16*) are also shown (+). The solid line assumes that each H atom can reversibly bind with one Li atom.

Hard Carbon Precursors—Region 3

A number of carbons with voltage profiles similar to that in Fig. 2C have been reported. Materials that show a low voltage plateau with a capacity of several hundred milliampere-hours per gram and little hysteresis have been prepared by Omaru *et al.* (*21*) from pyrolyzed polyfurfuryl alcohol, by Takahashi *et al.* (*22*) from unspecified precursors, by Sonobe *et al.* (*23*) from pyrolyzed petroleum pitch, and by Liu *et al.* (*10*) from pyrolyzed epoxy novolac resin. Ishikawa *et al.* (*24*) believe that such a capacity corresponds to the filling of micropores in the carbon by clusters of lithium. This mechanism would be expected to give weakly bound lithium (relative to that in Li metal) and hence a very low voltage plateau, consistent with experimental results (Fig. 2C).

In our earlier work (*10*), we showed that these carbon materials consisted primarily of small single layers of carbon arranged more or less like a house of cards. We proposed that lithium could be adsorbed on both of the surfaces of these single sheets, leading to more lithium per carbon than in intercalated graphite. In lithium-intercalated graphite, there is one layer of intercalated lithium for each carbon sheet. In carbons composed mainly of single layers, Li atoms at the same areal density presumably could be adsorbed on each side of the sheet, leading to two layers of lithium for each carbon sheet and hence a theoretical maximum capacity of Li_2C_6 or 740 mA·hour g^{-1}. To test this hypothesis, we prepared a series of carbons with varying fractions of single layers (*10*) and measured how much lithium could be inserted within them. Figure 5 shows that the reversible specific capacity of these region 3 materials increases with their single-layer fraction. Small-angle x-ray scattering (SAX) showed that each of the materials with a capacity of >400 mA·hour g^{-1} in Fig. 5 contained appreciable nanoporosity (*10*).

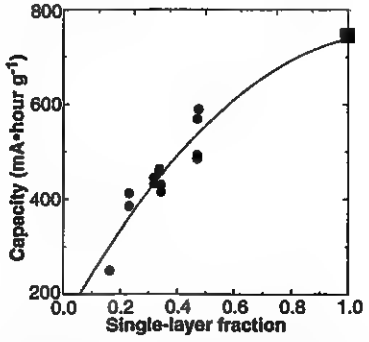

Fig. 5. Reversible capacity of region 3 carbons prepared from epoxy-novolac resins heated between 900° and 1100°C plotted as a function of their single-layer fraction. The square point is that hypothesized for complete adsorption on both sides of a carbon consisting only of single layers, and the line is a guide for the eye.

It is easy to reconcile the surface adsorption model proposed here with the pore-filling model of Ishikawa *et al.* (*24*). When the pores become small enough (SAX shows that they are on the order of 15 Å in diameter), the adsorption of lithium on the pore surfaces will essentially fill the pores entirely. In both models, we expect the lithium to be weakly bound (relative to that in Li metal), consistent with the low voltage plateau in Fig. 2C. In the sample used in Fig. 2C, ~50% of the C atoms were in single layers, with the remainder in double- and triple-layer groupings. The steeply sloping portion of the voltage profile in Fig. 2C is thought to arise from the insertion of lithium between carbon layers that are stacked in a roughly parallel fashion.

Conclusions

Three different mechanisms appear to be responsible for the insertion of lithium in carbonaceous materials that are relevant to the battery industry. In graphitic carbons (region 1), intercalation occurs, but lithium cannot be accommodated between adjacent carbon layers that show turbostratic disorder. In carbons that contain substantial amounts of hydrogen (region 2), the maximum amount of lithium that can be inserted is proportional to the hydrogen content, which suggests that the lithium binds somehow in the vicinity of the H atoms. The voltage-capacity behavior of the hydrogen-containing carbons shows large hysteresis. In materials that are made up predominantly of single layers of carbon and hence inherently incorporate nanopores (region 3), lithium appears to adsorb on both sides of the carbon layers. The work presented here and in (*8–10*) qualitatively identifies the dominant physical mechanisms for the reaction of lithium with the important carbon types. Substantial experimental and theoretical work is needed before these mechanisms can be considered proven.

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Neurotrophins and Neuronal Plasticity

Hans Thoenen

There is increasing evidence that neurotrophins (NTs) are involved in processes of neuronal plasticity besides their well-established actions in regulating the survival, differentiation, and maintenance of functions of specific populations of neurons. Nerve growth factor, brain-derived neurotrophic factor, NT-4/5, and corresponding antibodies dramatically modify the development of the visual cortex. Although the neuronal elements involved have not yet been identified, complementary studies of other systems have demonstrated that NT synthesis is rapidly regulated by neuronal activity and that NTs are released in an activity-dependent manner from neuronal dendrites. These data, together with the observation that NTs enhance transmitter release from neurons that express the corresponding signal-transducing Trk receptors, suggest a role for NTs as selective retrograde messengers that regulate synaptic efficacy.

Neurotrophic factors have been considered predominantly with respect to their functions in regulating the survival and differentiation of selective populations of neurons during embryonic development and the maintenance of specific functions of those neurons in adulthood (*1*). The spectrum of the biological actions of neurotrophins (NTs) [the general term coined for members of the nerve growth factor (NGF) gene family] is determined by the site and extent of their expression (*2*) and of the expression of the corresponding receptors (*1*, *3*) (Fig. 1). The delicate equilibrium between the availability of NTs and the survival and maintenance of specific populations of neurons becomes impressively apparent in NT knockout mice (*4–7*). For instance, in mice in which the gene encoding NT-3 has been targeted by homologous recombination, the inactivation of even one allele of the NT-3 gene reduces NT-3 mRNA amounts by about half and results in a massive reduction of cutaneous mechanoreceptors and of the corresponding end organs, the Merkel cells (*8*). Accordingly, in the peripheral sensory and sympathetic nervous systems, specific populations of neurons are supported by specific NTs, and the inactivation of the genes encoding them results in serious characteristic defects of the sensory and sympathetic nervous systems (*4–7*). In contrast, in the central nervous system (CNS) there is much overlap in the trophic support of individual neurons (*9*, *10*). The disruption of the expression of an individual factor by gene targeting does not result in changes as dramatic as those in the periphery (*4–7*). Therefore, the results of NT gene targeting experiments in the CNS may, at first sight, appear disappointing, showing at best a reduced expression of choline acetyltransferase (*4*) or of selective neuropeptides and calcium-binding proteins (*6*), which are known to be regulated by different NTs (*6*, *9*, *11*, *12*). However, such gene targeting experiments offer the possibility of identifying subtle NT effects that refine neuronal functions, such as activity-dependent neuronal plasticity. "Neuronal plasticity" is used to describe a great variety of changes in neuronal structure and function, but its use here is confined exclusively to activity-dependent, prolonged functional changes, accompanied by corresponding biochemical and possibly morphological alterations. Indeed, there is increasing evidence that NTs are involved in specific aspects of neuronal plasticity. This evidence originates from observations made in complex integrated systems in vivo, in particular of the influence of prolonged administration of NTs and of corresponding antibodies (Abs) on the development of the rat and cat visual cortex, the most extensively studied and well-understood area of the mammalian cortex (*13*). Although these observations did not reveal the underlying mechanisms, including the identification of individual neurons involved in NT-mediated plasticity, they nevertheless made NTs attractive candidates for the performance of essential roles in the development and activity-dependent modification of neuronal circuits. However, increasingly detailed analyses of the activity-dependent regulation of NT synthesis (*9*), the mechanism and site of NT release from neurons (*14*, *15*), and the presynaptic modulation by NTs of the release of classical transmitters (*15–19*) have recently appeared, which are promising with regard to development of a molecular understanding of the more complex integrated systems of established physiological relevance.

Activity-Dependent Regulation of NT Synthesis in the CNS

In contrast to the peripheral nervous system, in the CNS NTs are synthesized predominantly by neurons under physiological conditions (*2*, *9*) and, at least in the case of brain-derived neurotrophic factor (BDNF) and NGF, the amounts of these NT mRNAs are regulated by neuronal activity (*9*, *20*) in addition to hormonal influences (*9*, *11*, *21*). Combinations of in vitro and in vivo analyses, performed mostly in the rat hippocampus and cerebral cortex, have demonstrated that the activity-dependent regulation is mediated by classical neurotransmitters. Up-regulation is effected by glutamate via *N*-methyl-D-aspar-

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tate (NMDA) and non-NMDA receptors and, with different relative prevalence during development, also by acetylcholine via muscarinic receptors (9, 22). Down-regulation is mediated predominantly by γ-aminobutyric acid (GABA) via $GABA_A$ receptors (9, 22, 23). More important, it has become apparent that this activity-dependent regulation not only functions under extreme experimental conditions, such as initiation of seizures (20, 24), but that these regulatory mechanisms also are involved in the maintenance of normal physiological amounts of NGF and BDNF (22). However, neither NT-3 nor NT-4/5 is directly regulated by neuronal activity (9). Moreover, the synthesis of NTs, in particular BDNF, is regulated by physiological stimuli such as visual input (25). Specifically, blockade of sensory input to the visual cortex by intraocular injection of tetrodotoxin or by dark-rearing results in a rapid down-regulation of BDNF mRNA (25), and exposure of dark-reared animals to light rapidly restores the layer-specific BDNF mRNA amounts in the rat visual cortex (25). Because dark-rearing dramatically influences the functional development of the visual cortex (26), it is of particular interest that, when newborn rats are raised in the dark, the developmental increase of BDNF in the visual cortex is reduced, indicating that visual input is essential for the developmental regulation of BDNF in the visual system (25). Further examples of highly specific regulation of NT synthesis by physiological or subtle experimental stimuli are the up-regulation of BDNF in the paraventricular nucleus by osmotic stress (27) and by stimuli designed to initiate long-term potentiation (LTP) (28).

Activity-Dependent Secretion of NTs and Sites of Release

In the context of the evaluation of the potential functions of NTs in neuronal plasticity, it is important to characterize NT secretion from neurons. In the periphery, NGF is synthesized in a great variety of nonneuronal cells (1, 2, 29). The regulation of synthesis and release is independent of neuronal input (30). NGF is secreted according to the constitutive calcium-independent pathway (31). In the CNS, as first demonstrated for NGF (14, 15) and recently also for BDNF (32), NTs are secreted by neurons in both constitutive and activity-dependent pathways. In hippocampal slices and primary cultures of hippocampal neurons, the activity-dependent secretion of NTs initiated by high potassium or by veratridine, glutamate, or carbachol depends on extracellular sodium but is independent of extracellular calcium (14, 15, 32). However, it does depend on intact intracellular calcium stores, and their depletion by thapsigargin or blockade by dantrolene results in a drastic reduction of activity-dependent NT release, which is also blocked by the high-affinity calcium chelator 1,2-bis(2-aminophenoxy) ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid, tetra(acetoxymethyl)-ester (BAPTA-AM), which penetrates the plasma membrane (14, 32) and neutralizes the action of calcium released from intracellular calcium stores. Thus, the activity-mediated sodium-dependent secretion of BDNF and NGF is unusual and shows characteristics that are distinctly different from those of the regulated secretion of neurotransmitters and neuropeptides (33) and the activity-dependent secretion of other proteins, such as acetylcholinesterase (34).

Immunohistochemical localization of NGF by confocal microscopy has demonstrated that NGF is not only localized in the perikaryon but also in all neuronal processes (15). The pattern of distribution is compatible with storage of NGF in endoplasmic reticulum–like compartments. However, the resolution attainable by light microscopy is unsatisfactory, and ultrastructural analysis remains mandatory in order to delineate the relation between the storage compartments from which NTs are released and the internal (intact) calcium stores that are necessary for activity-dependent NT release (14, 15, 32).

After more precise information on the ultrastructural localization of NTs is established, further analyses will examine their possible co-localization with other well-characterized secretory molecules, such as secretogranin-II. Abs to secretogranin-II stain not only large dense-core vesicles but also dendrites (which do not contain large dense-core vesicles) of hippocampal neurons (35). Moreover, in spite of the unconventional characteristics of the activity-dependent NT release mechanism, it nevertheless seems to share mechanisms with well-established, conventional regulated secretion (33). For instance, in preliminary experiments it has been demonstrated that neuronal NGF secretion is stimulated by cyclic adenosine 3′,5′-monophosphate (cAMP) derivatives, which penetrate the cell membrane (36). Conversely, cAMP antagonists interfere with activity-dependent release, and an involvement of synaptobrevin or synaptobrevin-like molecules (37) can be deduced from the observation that tetanus toxin also blocks the activity-dependent secretion of NGF (36).

It recently has become possible to identify the sites of activity-dependent NGF release (15). A high-affinity monoclonal antibody (mAb) that exclusively recognizes the nondenatured, biologically active NGF (14, 15) was used to reveal the sites of release after depolarization of cultured hippocampal neurons by high potassium or by glutamate (Fig. 2). Given that BDNF shows the same secretion characteristic (32) as NGF, it is expected that the sites of release are also very similar.

Enhanced Release of Classical Neurotransmitters by NTs

In order to more precisely understand the function of NTs in neuronal plasticity, a comprehensive evaluation of the effects of neuronally released NTs is mandatory. In this context, discussion of the different mechanisms involved in prolonged facilitation of neurotransmission (that is, LTP) (28) is of particular interest. The existence and identity of the putative "retrograde factor" or factors released as a result of postsynaptic activation are a matter of controversy (38). The different NTs have specific pat-

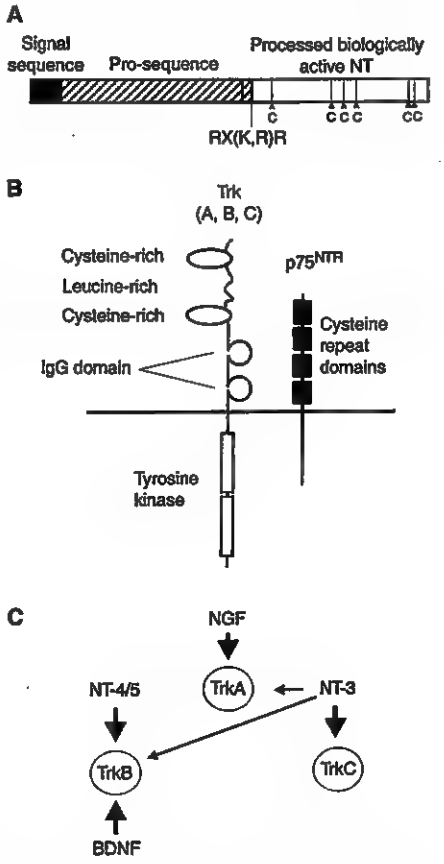

Fig. 1. NTs and their receptors. All the members of the NGF gene family (NTs) are synthesized as precursors (**A**) and are processed at classical dibasic cleavage sites into mature biologically active NTs that contain about 50% conserved domains. Those domains are mainly arranged around the strictly conserved cysteine (C) residues, which form three disulfide bridges that are essential for the three-dimensional structure and biological activity of NTs (1). K, lysine; R, arginine. The NT effects are mediated via Trk (A, B, or C) tyrosine kinase receptors and by $p75^{NTR}$ (**B**). The individual NTs (NGF, BDNF, NT-3, and NT-4/5) are assigned to individual Trk receptors (**C**). All NTs have the same affinity to $p75^{NTR}$, which enhances the affinity and increases the specificity of the individual NTs to their Trk receptors (3). The significance of the Trk-independent signal transduction via $p75^{NTR}$ is a matter of controversy and intense research (3).

terns of expression, as do the corresponding Trk receptors (*2*, *3*), and therefore NTs cannot be the retrograde messengers that enhance synaptic transmission in general, as is postulated for NO, CO, and arachidonic acid (*28*, *38*). However, NTs might have a more limited and specific function as retrograde modulators. Indeed, in co-cultures of embryonic *Xenopus* spinal neurons with myotubes, the administration of BDNF and NT-3, but not NGF, initiates an increase in spontaneous synaptic currents, reflecting an enhanced acetylcholine release from presynaptic terminals (*16*). That NTs have similar effects in the CNS can be deduced from observations that, in synaptosomal preparations of the rat hippocampus, NTs enhance the release of acetylcholine and glutamate (*17*), and that BDNF and NT-3 enhance spontaneous synaptic activity in primary cultures of hippocampal neurons (*18*) and also enhance the synaptic transmission evolving from the stimulation of the Schaffer collaterals in hippocampal slices (*19*). Injection of NTs into the hippocampus of behaving rats immediately initiated characteristic electrical activities resulting from neurotransmitter release (*15*, *39*). The electrical activity started at the injection site and propagated to the contralateral hippocampus and then to both cortices (*39*). The effects initiated by NGF were predominantly cholinergic in nature and could be blocked by muscarinic receptor antagonists. For BDNF, the glutamatergic effects dominated and could be blocked by corresponding antagonists (*39*). All of these observations indicate an enhancement of transmitter release from presynaptic nerve terminals that presumably express the appropriate Trk receptors. As is consistent with this interpretation, all of these effects can be blocked by K252A, a tyrosine-kinase inhibitor with a preference, although not absolute specificity, for Trk receptor tyrosine kinases (*40*). The proposal that transmitter release is facilitated by NTs via presynaptic Trk receptors is further supported by the observation that NTs initiate an immediate increase in intracellular calcium concentrations (*15*, *41*, *42*), an increase that is dependent on extracellular calcium (*42*), which is a prerequisite for activity-dependent release of classical neurotransmitters (*33*). Interestingly, hippocampal neurons, kept in culture for several weeks, exhibit spontaneous oscillation of intracellular calcium concentrations in their dendritic processes. These periodic calcium transients are acutely enhanced by BDNF administration (Fig. 2).

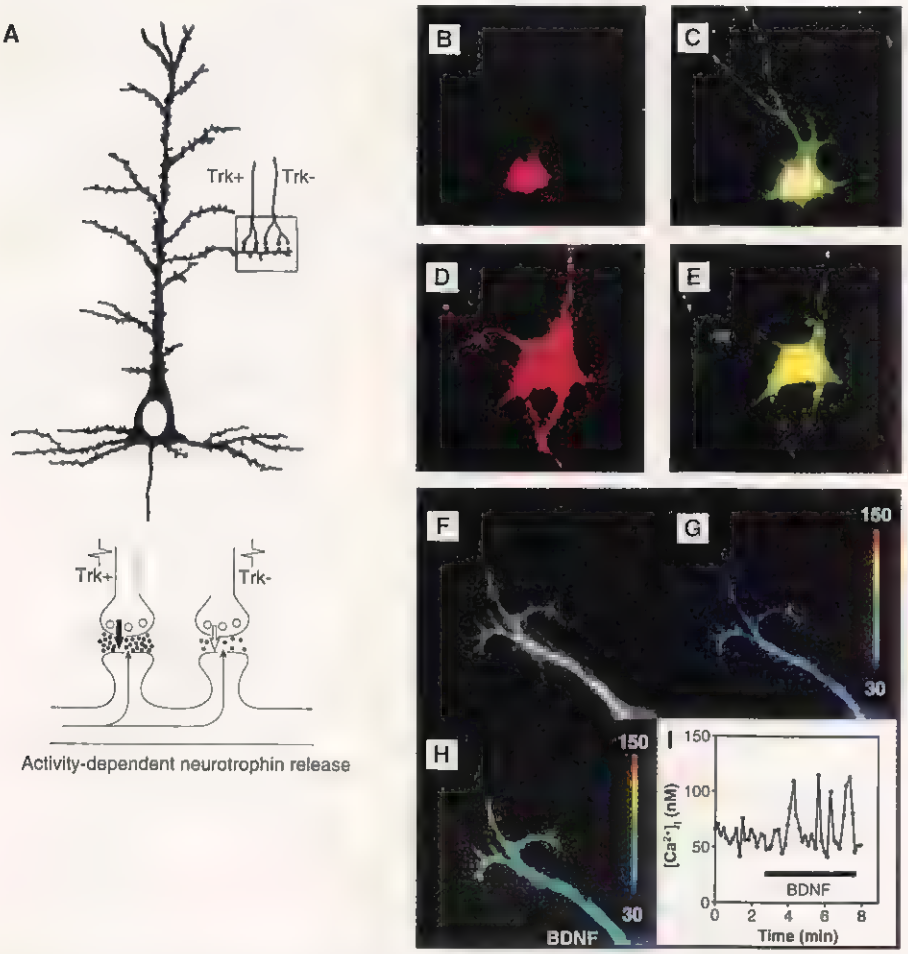

Fig. 2. Activity-dependent release of NTs from dendrites and their function as retrograde messengers. (**A**) Schematic presentation of NT-synthesizing neuron receiving input from a neuron that expresses Trk receptors at its synaptic endings (Trk+) and a neuron that does not (Trk−). Activity-dependent release of NT from the dendrite has a positive feedback effect on the Trk+ nerve terminal, enhancing the efficacy of this synapse (solid arrow, lower panel). (**B** to **E**) Constitutive and activity-dependent release of NGF from hippocampal neurons. Constitutive release, which can be blocked by lowering the temperature below 15°C (*14*, *15*), becomes apparent predominantly at the surface of the perikaryon (B). In contrast, glutamate-evoked NGF release occurs throughout the processes, including dendrites (D). All experiments were performed in hippocampal cultures that were previously transfected with an NGF construct containing a reporter gene [chloramphenicol acetyltransferase (CAT)] in order to identify the transfected neurons. The experimental procedures were performed as described (*14*, *15*). NGF at the cell surface was visualized in native unpermeabilized cells (*15*). After photographs were taken [(B) and (D)] the same preparation was permeabilized and the CAT reporter gene was identified [(C) and (E)]. (F) to (I) show enhancement of spontaneous fluctuations of calcium transients in a dendrite by BDNF in 3-week-old cultures of hippocampal neurons. These neurons, forming an extensive network of synapse-forming neuronal processes, were loaded with fura-2 (*15*, *41*). The changes in internal calcium concentrations $[Ca^{2+}]_i$ are indicated in nanomolar concentrations represented by a color scale [(G) and (H)] ranging from 30 to 150 nM. (**F**) Fluorescent picture of a fura-2–loaded neuron; the point of observation is boxed. (**G**) Calcium transients in the absence of BDNF. (**H**) Calcium transients in the presence of BDNF. (**I**) Time course of oscillating calcium transients in the boxed domain of the dendrite in the absence and presence of BDNF.

Involvement of NTs in Neuronal Plasticity in Organotypic Integrated Systems in Vitro

The NT-mediated enhanced release of conventional transmitters from neurons expressing the appropriate Trk receptors strongly suggests a function of NTs as retrograde messengers, enhancing synaptic efficacy in selective neuronal systems. Indeed, in hippocampal slices, exogenous administration of NTs resulted in enhanced synaptic efficacy in the CA3-CA1 system (*19*). Local administration of BDNF resulted in enhanced CA1 field potentials, induced by stimulation of Schaffer collaterals (*19*). Conversely, recent experiments have demonstrated that in BDNF knockout mice, the LTP in the same system is drastically reduced (*43*). The frequency with which a successful LTP response could be elicited was reduced from 90% in controls to 30% in BDNF-deficient animals. This LTP impairment was not restricted to homozygous mutant mice but was also present in heterozygotes (*43*), in which the hippocampal

BDNF mRNA amounts were reduced to about 50%, demonstrating that gene dosage–dependent reductions of NTs may result in distinct functional impairments in the CNS as well. This impairment of LTP became apparent while the basic pharmacological parameters of neuronal transmission remained unchanged (*43*). The specificity of the function of BDNF in the modulation of LTP is further supported by the recent observation that adenovirus-mediated BDNF gene transfer into CA1 neurons of hippocampal slices of BDNF knockout mice restored LTP in this system (*44*).

Involvement of NTs in Neuronal Plasticity of the Visual Cortex in Vivo

The information collected from the experimental systems summarized above is compatible with the interpretation that NTs may be essential for neuronal plasticity. In spite of gaps in the detailed understanding of the molecular mechanisms, information that is already available may contribute to understanding of the spectacular changes that result from the prolonged administration of NTs to the visual cortex of rats (*13*, *45*) and cats (*46*–*48*) at different developmental stages. Connectivity in the visual cortex can be manipulated by change in the pattern of visual input—for instance, by monocular deprivation during restricted periods of development (*49*). Monocular deprivation renders neurons of the visual cortex, most of which are normally binocular, nonresponsive to stimuli presented to the deprived eye (ocular dominance shift) (Fig. 3). The expansion of the connections contributed by the nondeprived eye depends critically on the activation of neurons via NMDA receptors (*50*), and it also is modulated by cholinergic, serotoninergic, and adrenergic mechanisms (*51*). Maffei and coworkers (*45*) demonstrated that in rats the intraventricular injection of NGF during the critical period of ocular dominance plasticity, when cortical neurons are sensitive to manipulations of visual experience, prevented the effect of monocular deprivation (Fig. 3). One possible interpretation is that NGF affects the development of cortical neurons by interfering with normal synaptic transmission. However, because other visual cortical functions such as visual acuity and orientational selectivity (*52*) developed normally in these animals, it seems more likely that NGF precociously stabilizes the neuronal connections that are responsible for the binocular response. Recently, Cabelli and co-workers reported that in developing kittens the local infusion of BDNF and NT-4/5 (but not NT-3 and NGF) prevented the formation of ocular dominance columns (*47*). Whether this process is due to a sprouting of geniculocortical afferents or to a blockade of normal development is not yet known.

The dramatic effects of NTs on the visual system are of particular interest, as this system can be manipulated experimentally in a very precise manner (*53*) by changes in the visual input (*49*, *53*). Although the observations made do not permit an understanding of the underlying mechanisms, the physiological relevance is supported by recent reports from the laboratories of Maffei and Shatz that blockade of endogenous NTs by mAbs to NGF or by TrkB immunoglobulin G (IgG) fusion proteins (which block the effects of BDNF and NT-4/5) had dramatic effects on the development of the visual cortex (*54*, *55*). The implantation of hybridoma cells secreting blocking Abs to NGF into the lateral ventricle of rats in the postcritical period (Fig. 3) resulted in an extension of the time period during which the binocularity of visual cortical neurons can still be influenced by monocular deprivation (*54*). This situation is a mirror image of the effects of the intraventricular administration of NGF during the critical period (*45*) (Fig. 3). Moreover, the neutralization of endogenous NGF by mAbs also resulted in a reduction in the size of the neuronal cell bodies of the lateral geniculate body that project to the visual cortex (*54*). However, this seems to be an indirect effect, because these neurons do not express TrkA receptors and, accordingly, labeled NGF injected into the rat visual cortex is not retrogradely transported to the cell bodies in the lateral geniculate body (*56*). Moreover, the prolonged administration of NGF did not result in hypertrophy of the geniculate neurons, as has been observed for NGF-responsive neurons in the septum and striatum and other brain areas (*57*). Cholinergic basal forebrain neurons that project to the visual cortex and are responsive to NGF [through expression of TrkA and p75 neurotrophin receptor (NTR)] seem to be unlikely to play an essential role in the modulation of exogenous and endogenous NGF to influence visual cortex plasticity, because interruption of the connections between the cholinergic basal forebrain neurons and the visual cortex (abolishing virtually com-

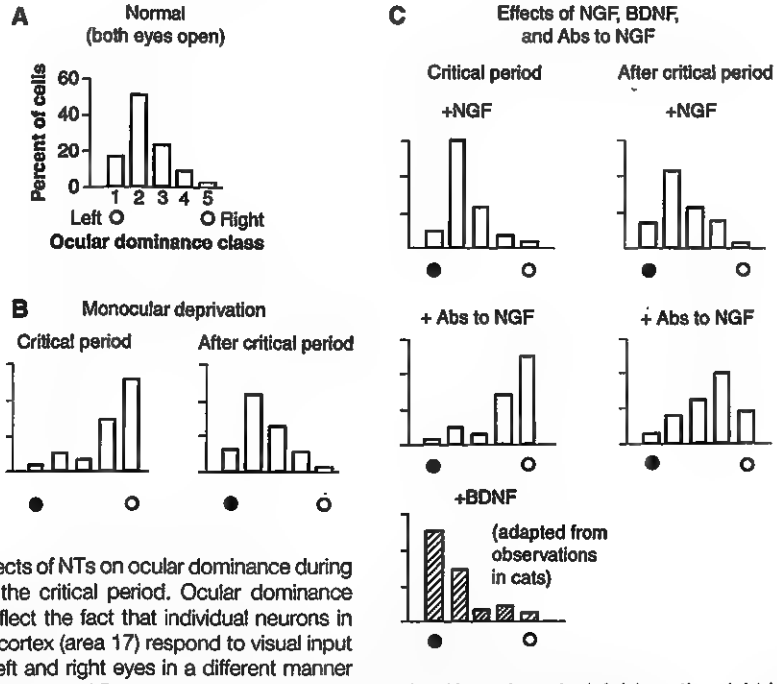

Fig. 3. Effects of NTs on ocular dominance during and after the critical period. Ocular dominance classes reflect the fact that individual neurons in the visual cortex (area 17) respond to visual input from the left and right eyes in a different manner (*49*, *64*). Class 1 and 5 neurons respond exclusively to visual input from the left (class 1) or right (class 5) eye. Class 2 and 4 neurons respond to visual input from both left and right eyes, but with a preference for the left (class 2) or right (class 4) eye. Neurons of class 3 respond strictly binocularly—that is, they respond equally to visual input from right and left eyes (*64*). Besides the classification from 1 to 5, which has been chosen for reasons of simplicity, there are also more subtle classifications from 1 to 7 (*49*). **(A)** During the critical period [in rats from about postnatal day (P) 15 to 45 and in cats from about P28 to P72], the responsiveness of these neurons can be shifted so that the response is turned toward the open eye, and this shift in responsiveness remains fixed after reopening of the eye after the critical period. **(B)** Data analyzing the effects of NGF and Abs to NGF on the changes in ocular dominance caused by monocular deprivation of rats are adapted from references (*45*, *54*). **(C)** The side of eye closure is indicated by filled circles. Because no data on the effects of BDNF in rats are available, data obtained in cats (*48*) were adapted to those in rats, with the assumption that BDNF would have similar effects in rats. This seems to be justified, because the effects of NGF in cats appear to correspond to those in rats, at least with respect to visual acuity (*46*).

pletely the retrograde labeling of septal cholinergic neurons from the visual cortex) did not mimic the effects of Abs to NGF on the morphology of the lateral geniculate neurons (*13*). However, it should be borne in mind that in cats the extensive cytotoxic destruction of basal forebrain neurons, resulting in a massive decrease of choline acetyltransferase activity in the visual cortex, had no effect on ocular dominance plasticity, whereas blockade of muscarinic receptors prevented ocular dominance changes (*51*), indicating that, under these experimental conditions, ocular dominance plasticity is only abolished if the cholinergic block is complete.

The local administration of BDNF to the visual cortex of kittens during the critical period prevented the formation of ocular dominance columns (*47*) and produced a paradoxical shift of the responsiveness of cat visual cortical neurons toward the deprived eye (*48*) (Fig. 3). The blockade of the formation of ocular dominance columns by BDNF infusion after monocular deprivation is thought to result from interference with the normal activity-dependent competition for BDNF by neurons (*53*) that project from the lateral geniculate nucleus to spiny neurons in layer IV. Unexpectedly, in the rat visual cortex, layer IV neurons contain particularly small amounts of BDNF mRNA and do not exhibit light-induced regulation of BDNF mRNA synthesis (*25*). Activity-dependent competition includes a variety of possible mechanisms, such as activity-dependent local release, activity-dependent regulation of synthesis, or activity-dependent uptake of BDNF. However, in the periphery, no enhancement of the retrograde transport of NGF in sympathetic neurons could be demonstrated by an augmented neuronal activity (*58*). The preventive effects of local infusion of BDNF and NT-4/5 (*47*), as well as the paradoxical shift in the cat visual cortex after monocular deprivation (*48*), alternatively may result from an activation of GABA-containing interneurons in the visual cortex. In rodents, cortical GABA-containing neurons express TrkB receptors, and the observed effects could be due to augmented GABA synthesis, together with a sprouting of these interneurons and an enhanced release of GABA, in analogy to the enhancement of neurotransmitter release from cholinergic and glutamatergic neurons (*15–18*, *39*) by NTs acting via the corresponding Trk receptors. This interpretation is supported by the fact that the infusion of BDNF into the visual cortex of monocularly deprived kittens had a similar effect, as had been shown in previous experiments to be caused by the infusion of the $GABA_A$ receptor agonist muscimol (*59*).

Conclusions and Perspectives

The evidence for the involvement of NTs in neuronal plasticity evolves from observations made in complex integrated neuronal systems in vivo and from detailed analyses in a variety of in vitro systems. The local administration of NTs (*13*, *45*, *47*, *48*) and molecules that neutralize the action of NTs (*13*, *54*, *55*) (blocking Abs or Trk-IgG fusion proteins) to the visual cortex of rats or cats changes the structural and functional development of the visual cortex in a dramatic manner. Most important, the continuous release of blocking Abs to NGF from implanted hybridoma cells not only antagonizes the effects of exogenous NGF but also dramatically influences the normal development of ocular dominance in a manner that is a mirror image of that of exogenously administered NGF (Fig. 3). Moreover, the development of ocular dominance columns in kittens by means of monocular deprivation is not only prevented by the local infusion of BDNF and NT-4/5 (*47*), but also by TrkB-IgG, which blocks the effect of BDNF and NT-4/5 (*55*). Thus, the effects of NTs do not only represent pharmacological actions, but clearly indicate that NTs play an essential physiological role in the activity-dependent development of the visual cortex. A major deficit in the analysis of NT function in visual cortex plasticity is the lack of precise information about which neurons are involved in the synthesis of NTs and which are the neuronal elements that respond to exogenous and (more importantly) endogenous NTs.

NGF and BDNF mRNAs are regulated in a highly specific manner by physiological stimuli in different areas of the CNS (*9*, *22*, *25*, *27*, *28*). In contrast to the situation in the periphery, they are not only released constitutively (*31*) but also by means of an unconventional activity-dependent mechanism (*14*, *15*, *32*). The sites of activity-dependent release include dendrites (*15*). More detailed information on the precise ultrastructural localization of the storage sites of NTs and their relation to calcium stores, which have to be intact to mediate the sodium-dependent release of NTs, is needed. It will also be essential to evaluate whether localized release of NTs from restricted domains of dendrites or even individual spines is possible.

In addition to the localization of NTs and their receptors in integrated structures of interest, such as the visual cortex and the hippocampus, "second generation" knockout procedures seem promising. They should permit restricted inactivation of NTs and their receptors in subpopulations of neurons (*60*), for example, by using the different BDNF promoters (*61*) and the choline acetyltransferase promoter (*62*) for the transgenic expression of a specific recombinase as a prerequisite for tissue-specific gene inactivation (*60*). Moreover, preliminary experiments with gene transfer by adenoviruses (*44*) look promising for the controlled overexpression of NTs in integrated systems in vitro (primary cultures and slice preparations) and in vivo (*63*), thus opening up the possibility of locally interfering with the function of NTs by the use of antisense constructs or local expression of Abs to NT and Trk-IgG molecules. Thus, the transgenic approach, which so far has been restricted to mice, can be complemented by viral-mediated gene transfer in other species, not only for the local overexpression of NTs or inhibition of their synthesis by antisense constructs but also by the expression of blocking Abs directed against NTs or their receptors.

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RESEARCH ARTICLE

Biostratigraphic and Geochronologic Constraints on Early Animal Evolution

John P. Grotzinger, Samuel A. Bowring, Beverly Z. Saylor, Alan J. Kaufman

Two distinct evolutionary pulses, represented by the Vendian Ediacaran fauna and Cambrian small shelly faunas, are generally thought to characterize the emergence of macroscopic animals at the end of Precambrian time. Biostratigraphic and uranium-lead zircon age data from Namibia indicate that most globally distributed Ediacaran fossils are no older than 549 million years old and some are as young as 543 million years old, essentially coincident with the Precambrian-Cambrian boundary. These data suggest that the most diverse assemblages of Ediacaran animals existed within 6 million years of the Precambrian-Cambrian boundary and that simple discoid animals may have appeared at least 50 million years earlier.

Early animal evolution is widely thought to have occurred in two discrete steps, set apart by tens of millions of years. Evolutionary models have had to explain an initial episode at the end of Precambrian time (Vendian Period) in which simple, mostly soft-bodied, cnidarian-grade organisms, bilaterians, and problematica (collectively referred to as the Ediacaran fauna) first appeared, followed by a second phase early in the Cambrian Period in which small shelly invertebrates (*1*) and complex trace fossils appeared in the Nemakit-Daldyn stage and rapidly diversified during the subsequent Tommotian, Adtabanian, and Botomian stages (*2–8*). Because the affinities of the earlier, Ediacaran fauna are still debated (compare *5*, *9*, *10*), the apparent wide separation in time of these two evolutionary pulses has been used to support phylogenetic arguments that these creatures are not simple precursors to later forms, but instead

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represent a failed lineage, perhaps unrelated to the animal kingdom (*4–7, 11, 12*).

Temporal calibration of past evolutionary events, correlated using emerging biostratigraphic, chemostratigraphic, and magnetostratigraphic data sets, is possible only with precise absolute age control such as that offered by U-Pb zircon dating of volcanic rocks intercalated within sedimentary successions (*13–15*). In this article, we report new biostratigraphic and U-Pb radiometric age data from sedimentary and volcanic rocks of the Vendian-Cambrian Nama Group in southern Namibia. Biostratigraphic constraints are provided by specimens of the common Ediacaran taxon *Pteridinium* and a second frond-like form of uncertain affinity, complex spiral burrows of possibly late Vendian age, the Early Cambrian trace fossils *Phycodes pedum*, *P. coronatum*, and *Curvolithus*, and a new form of skeletalized fossil whose stratigraphic range is similar to that of *Cloudina*. Volcanic rocks were selected for dating that give the maximum age of the youngest Ediacaran fossils, the minimum duration of the Ediacaran fauna, the minimum age of the oldest skeletalized fossils, and the age of the Precambrian-Cambrian boundary. The dated sections have excellent chemostratigraphic tie points and fossil assemblages.

The Vendian time scale. For the Phanerozoic Eon, the geologic time scale primarily is based on a chronostratigraphy of biological events that are calibrated in absolute time with radiometric age determinations. The biostratigraphic basis for the Vendian time scale has been slow in developing. With the exception of simple, disk-shaped impressions of possible soft-bodied metazoans that occur in strata immediately below a Varanger-aged (*16*) glacial unit in the Mackenzie Mountains, Canada (*17*), all other occurrences of soft-bodied impressions are in strata younger than the Varanger glacial rocks (*18*). These younger impressions constitute the Ediacaran fauna, which was long thought to have been restricted to the first half of Vendian time. The apparent absence of Ediacaran fossils in younger Vendian strata provided the basis for erecting the Kotlin interval of the Vendian System (*16, 19, 20*). It has not been possible to recognize distinct biozones within Ediacaran-bearing strata because of taphonomic, paleoenvironmental, and possibly biogeographical factors (*3*), although recently it has been argued that some broad trends are apparent in their diversity (*21, 22*). Acritarchs may be useful in slightly older and younger rocks, but cannot be used to subdivide Ediacaran fossil ranges (*20*). Trace fossils and shelly fossils provide additional help but have low diversities throughout the Vendian (*23, 24*).

The carbon isotope record is proving to be critically important in unravelling Vendian history. As more and more sections are evaluated, it has become clear that the Vendian can be subdivided based on the oscillatory pattern of carbon isotope ratios (*18, 25*). As currently understood, the Vendian carbon isotope curve, beginning with the first rocks deposited above the Varanger glacial unit, features a shift to progressively lighter values and reaches a minimum ($\delta^{13}C_{carb}$ = −2 to −4 per mil relative to the PDB standard). It then increases to a maximum of about +4 to +5 per mil before decreasing to values that remain near 0 to +2 per mil until the close of Proterozoic time, when values decrease abruptly to −2 to −3 per mil just below the Precambrian-Cambrian boundary (*18, 21, 25, 26*). These excursions provide an independent framework against which to compare the temporal distribution of Ediacaran, trace fossil, and skeletal faunas.

Age estimates for the Vendian time scale and Precambrian-Cambrian boundary. Reliable age estimates for the Vendian time scale are sparse. Volcanic and intrusive rocks that underlie Varanger-age glaciogenic rocks are dated at 602 ± 3 Ma (million years ago) in Massachusetts (*27*) and 606 ± 3.7/− 2.9 in Newfoundland (*28*). We accept 600 Ma as the best maximum estimate for the age of the Varanger glacial event.

A volcanic ash bed interbedded with Ediacaran fossils in the Mistaken Point Formation of Newfoundland provides a direct U-Pb zircon age of 565 ± 3 Ma (*29*). Volcanic rocks of the Slawatycze Formation in Poland have a U-Pb zircon age of 551 ± 4 Ma (*30*), but provide only an indirect esti-

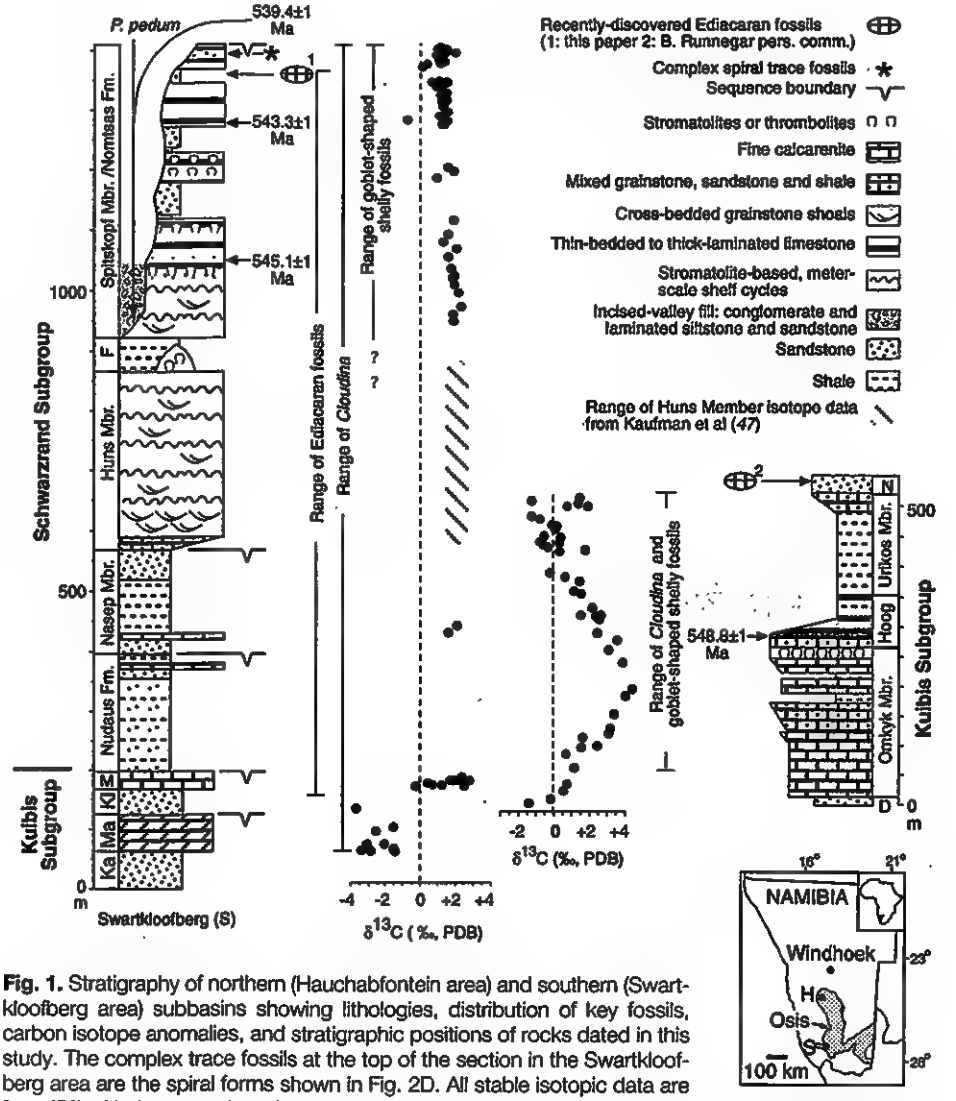

Fig. 1. Stratigraphy of northern (Hauchabfontein area) and southern (Swartkloofberg area) subbasins showing lithologies, distribution of key fossils, carbon isotope anomalies, and stratigraphic positions of rocks dated in this study. The complex trace fossils at the top of the section in the Swartkloofberg area are the spiral forms shown in Fig. 2D. All stable isotopic data are from (*50*) with the exception of the diagonally hachured band, which are from (*47*). Inset shows distribution of Nama Group outcrop (gray) and locations of Hauchabfontein and Swartkloofberg areas in southern Namibia. Ka, Kanies Member; Ma, Mara Member; Kl, Kliphoek Member; M, Mooifontein Member; F, Feldschuhhorn Member; D, Dabis Formation; Hoog, Hoogland Member; N, Niederhagen Member (Schwarzrand Subgroup); and H, Hauchabfontein.

mate; the Slawatycze Formation is known exclusively from subsurface drill-hole data and is thought to correlate lithologically with the volcanogenic Volhyn Group in the Ukraine, which, in turn, is overlain by the Ediacaran-fossil bearing Redkino interval (*30*, *31*).

The Precambrian-Cambrian boundary is defined as the point in rock at a section located in southeastern Newfoundland where the trace fossil *Phycodes pedum* first appears (*32*). However, no volcanic rocks are present at or in close proximity to the boundary (*32*, *33*). Consequently, the age of the boundary can be calibrated only through correlation with other reference sections that do contain datable volcanic rocks. Over the past decade, estimates of the absolute age of the Precambrian-Cambrian boundary have ranged from 600 to 530 Ma (*13*, *34*, *35*). Previous U-Pb zircon studies constrain the age of the Precambrian-Cambrian boundary to be younger than about 550 Ma and older than about 544 Ma (*13*, *36*).

The best minimum age estimates for the boundary come from ion-microprobe U-Pb studies of volcanic zircons from Lower Cambrian (Tommotian and younger) sections in South Australia, Morocco, and China (*14*, *37*) and from conventional zircon studies of volcanic rocks in Siberia and New Brunswick (*13*, *38*). Isachsen *et al.* (*38*) reported an age of 531 ± 1 Ma for a volcanic ash just below the Nemakit-Daldyn–Tommotian boundary in New Brunswick. Bowring *et al.* (*13*) obtained an upper intercept age of 543.8 + 5.1/−1.3 Ma (mean $^{207}Pb/^{206}Pb$ age of 543.9 ± 0.2 Ma) for a volcanic breccia intercalated within (*39*) basal Nemakit-Daldyn strata in northeastern Siberia and suggested that the age of the boundary is close to 544 Ma.

The best constraints on the maximum age of the boundary are given by U-Pb zircon ages of 560 ± 1 Ma for the Ercall granophyre of England (*40*), which is unconformably overlain by late Tommotian or early Atdabanian strata, and 551.4 ± 5.8 Ma for a rhyolite flow which lies well below the boundary at Fortune Bay, Newfoundland (*36*).

Previous studies of the age of the Nama Group provide only broad limits. A K-Ar study of detrital white micas from the Nama Group (*41*) led to the conclusion that the upper Nama Group is younger than the youngest detrital white micas (570 Ma) but older than low grade thermal alteration of the upper Nama Group at 530 to 500 Ma.

Vendian to Cambrian Nama Group, Namibia. The Vendian to Cambrian Nama Group is a >3000-m-thick succession of shallow marine and fluvial, siliciclastic and carbonate sedimentary rocks located in southern Namibia (Fig. 1). The Nama Group contains globally recognized Vendian and Cambrian body-fossil, microfossil, and trace-fossil assemblages as well as endemic biotas (*23*, *42*–*46*). Carbonate rocks are abundant throughout most of the Nama Group and their carbon-isotopic variability (*47*) compares well with that from Vendian sections on other continents (*18*, *25*). The Nama basin is partitioned into northern and southern subbasins, set apart by the intervening Osis arch, across which most stratigraphic units thin (*48*, *49*). We identified and selected several ash beds for dating on the basis of their strategic stratigraphic positions.

Northern subbasin. One ash bed, 94-N-10b, was collected from the Kuibis Subgroup in the northern subbasin (Fig. 1). The Kuibis Subgroup thickens northward from the Osis arch to more than 400 m near Hauchabfontein, where the 30 cm-thick ash bed lies in the Hoogland Member, 270 m above the base of the Kuibis Subgroup. At Hauchabfontein, the Kuibis Subgroup comprises a basal few meters of coarse, transgressive sandstone overlain by shallow marine carbonate platform deposits (*50*) (Fig. 1). Deep-water limestone rhythmite in the Hoogland Member grades upward to shale, which dominates the upper 100 m of the Kuibis Subgroup. The overlying Schwarzrand Subgroup thickens northward from Osis to form a nearly 800 m-thick succession of alternating prodeltaic mudstone and tabular-bedded deltaic sandstone.

Carbon isotope data from the Kuibis Subgroup at Hauchabfontein reveal a positive excursion characterized by $\delta^{13}C$ values that increase upward monotonically from slightly negative at the base to a peak of nearly +5 per mil approximately 60 m below the Hoogland ash; values then decrease to an average of about 0 per mil (*50*) (Fig. 1). Skeletalized fossils occur throughout the Kuibis Subgroup and include *Cloudina* and a previously unreported goblet-shaped form (Fig. 2A). Upward in the section, the Ediacaran fossil *Pteridinium* occurs in the Niederhagen Member of the basal Schwarzrand Subgroup (*51*), and distinctive acritarchs occur in overlying shales (*44*). The carbon isotope pattern and assemblage of fossils are consistent with a Vendian age for at least the Kuibis and lower Schwarzrand subgroups in the northern subbasin.

Southern subbasin. Three volcanic ashes were sampled in the southern subbasin (Fig. 1). The stratigraphically lowest is a 50-cm-

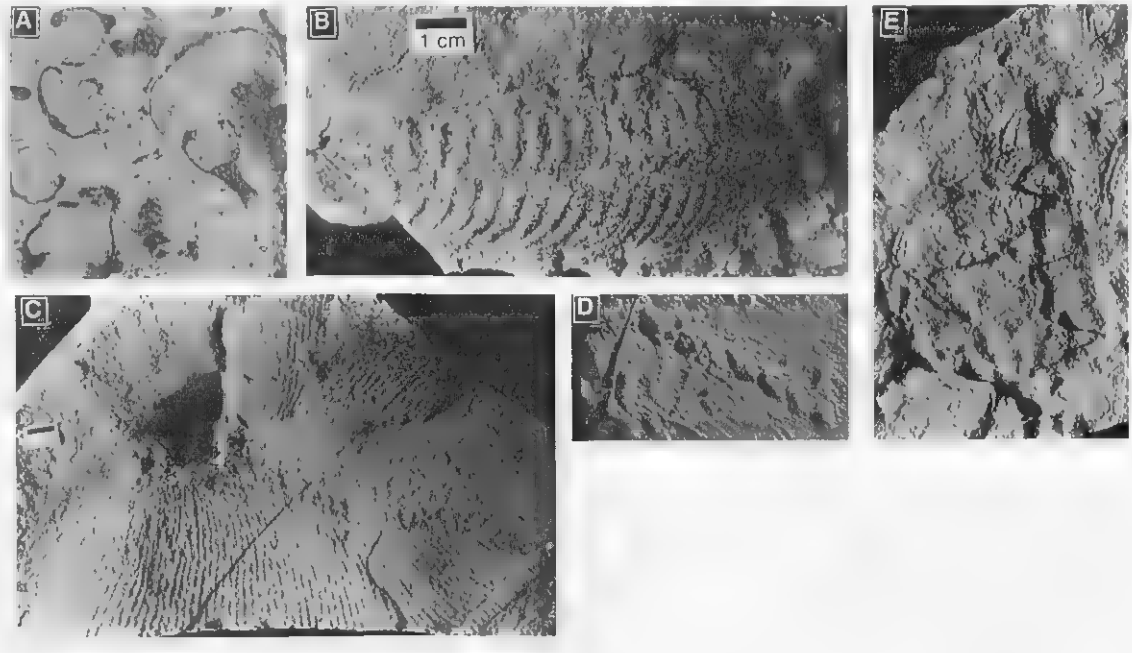

Fig. 2. New fossils from Nama Group. (**A**) Vase-shaped shelly fossils from middle Omkyk Member, Hauchabfontein area. Specimen at right-central part of photograph is approximately 1 cm long. (**B**) *Ptendinium* from upper Spitskopf Member, Swartkloofberg area. (**C**) Possible *Nasepia* or new Dickinsonid-like form from upper Spitskopf Member, Swartkloofberg area. (**D**) Spiral burrow, uppermost Spitskopf Member, Swartkloofberg area. Specimen is approximately 2 cm long. (**E**) *Curvolithus* and *Phycodes pedum* (bottom of photograph) from the incised valley fill of the basal Nomtsas Formation, Sonntagsbrun area. Width of photograph is about 4 cm.

thick bed (sample BZS-7) in the lower part of the Spitskopf Member at Witputs. A second ash (sample 94-N-11), 20 cm thick, is exposed in the uppermost Spitskopf at Swartpunt, 135 m above the lower Spitskopf ash. The third, stratigraphically highest ash (sample 92-N-2) is exposed in the lowermost Nomtsas Formation at Swartkloofberg, a few meters above the sequence boundary that includes the Precambrian-Cambrian boundary.

The Kuibis and Schwarzrand subgroups in the southern subbasin thicken southwestward from the Osis arch to more than 1200 m near Swartkloofberg, where the thickest section is exposed (Fig. 1). The Kuibis Subgroup comprises two depositional sequences, each with basal fluvial to marginal marine sandstone overlain by subtidal carbonate rocks (*49*). Fine-grained, siliciclastic rocks deposited in a shelf and deltaic environment in the lower Schwarzrand Subgroup form two depositional sequences with a combined thickness of more than 350 m (*49*). The upper 600 m of the Schwarzrand Subgroup consists of shallow marine limestone (Huns Member), overlain by siliciclastic mudstone (Feldschuhhorn Member), in turn overlain by mixed deltaic to shallow marine siliciclastic-carbonate rocks (Spitskopf Member). The Spitskopf Member is overlain by the Nomtsas Formation, which forms the highest depositional sequence in the Schwarzrand Subgroup (*49*). The Spitskopf-Nomtsas contact is an erosional surface cut by incised valleys filled with fluvial to shallow-marine conglomerate, sandstone, and shale of the lower Nomtsas Formation (Fig. 1). This surface minimally extends from Swartkloofberg to Sonntagsbrunn, about 100 km to the east (*48*).

$\delta^{13}C$ values are negative in the lower Kuibis Subgroup and increase to values of about +4 per mil in the upper Kuibis Subgroup, similar to the pattern in the northern subbasin (*47*, *50*) (Fig. 1). Above the Kuibis Subgroup, $\delta^{13}C$ values begin near +2 per mil and remain relatively stable through the Huns Member (Kaufman *et al.* 1991) but systematically decrease through the Spitskopf Member to near +1 per mil at its top (*50*). The presence of this trend in the Spitskopf Member is consistent with a latest Vendian age for the top of the Spitskopf Member, as shown by recent work in northern Siberia (*52*). The lack of an overlying negative excursion, however, observed immediately beneath the Precambrian-Cambrian boundary at other localities (*21*, *25*, *52*, *53*), suggests that the youngest Vendian strata in the southern Nama Group either were removed along the sub-Nomtsas erosional surface or never deposited (*50*).

The oldest Ediacaran fossils in the Nama Group (*48*) occur just below the Mooifontein Member of the Kuibis Subgroup, where $\delta^{13}C$ values are high. We found the youngest Ediacaran fossils in the Nama Group near the top of the Spitskopf Member, 90 to 100 m above the upper Spitskopf ash (Fig. 1). Two frond-shaped forms are present (Fig. 2, B and C) including *Pteridinium* and a Dickinsonid-like fossil that may be *Nasepia* or possibly a new taxon (*54*).

The range of skeletalized fossils in the southern subbasin is similar to that of the Ediacaran soft-bodied fossils (Fig. 1). Occurrences of *Cloudina* extend from the lower Kuibis Subgroup through the Spitskopf Member of the Schwarzrand Subgroup (*48*). In addition, the same goblet-shaped skeletalized fossils found in the northern subbasin are also present in the south, extending at least from the top of the Huns Member through the top of the Spitskopf Member. Although previous reports indicate that *Cloudina*-bearing reefs occur in the lower Nomtsas Formation (*48*), it recently has been shown that these reefs actually occur at the top of the underlying Huns Member (*49*).

Simple trace fossils consistent with a Neoproterozoic age are found in the Schwarzrand Subgroup beneath the Nomtsas Formation (*42*, *48*). Complex, spiral trace fossils, possibly of late Vendian age, are seen at the top of the Spitskopf Member (Figs. 1 and 2D). The Cambrian index fossil *Phycodes pedum* was reported from the Nomtsas Formation at Swartkloofberg (*42*) (Fig. 1). We found *Phycodes pedum* in the Nomtsas Formation at Sonntagsbrunn along with the previously unreported Cambrian index fossils *Phycodes coronatum* and *Curvolithus* (Fig. 2E). Poorly preserved specimens of *Tricophycus pedum*, regarded as a Cambrian ichnofossil, recently have been reported from the middle Schwarzrand Subgroup (*55*). If this assignment is correct, then it suggests that this ichnogenera may have a range that extends below the Precambrian-Cambrian boundary, as recognized through chemostratigraphic and biostratigraphic correlations.

In summary, the chemostratigraphy and biostratigraphy of both the southern and northern subbasins of the Nama Group are similar to other Vendian successions around the world. The isotopically enriched carbonates of the Kuibis Subgroup, overlain by the thick Schwarzrand Subgroup containing relatively stable $\delta^{13}C$ values of +1 to +2 per mil, match well with the middle and upper segments of the composite Vendian carbon isotope curve (*18*, *25*). Significantly, many of the world's most diverse assemblages of Ediacaran fossils, including the type assemblage in the Ediacaran Hills of South Australia, occur in rocks deposited during the relatively stable $\delta^{13}C$ interval of +1 to +2 per mil that occurs below the Precambrian-Cambrian boundary (*21*, *25*, *26*). A final, brief negative shift, which occurs at the Precambrian-Cambrian boundary in several sections (*25*), is not present in Namibia, probably because the time that the negative excursion represents is incorporated within the hiatus represented by the Spitskopf-Nomtsas unconformity. Accordingly, the presence of trace fossils of the *Phycodes pedum* zone in strata immediately above the unconformity indicate an earliest Cambrian age for the basal Nomtsas Formation.

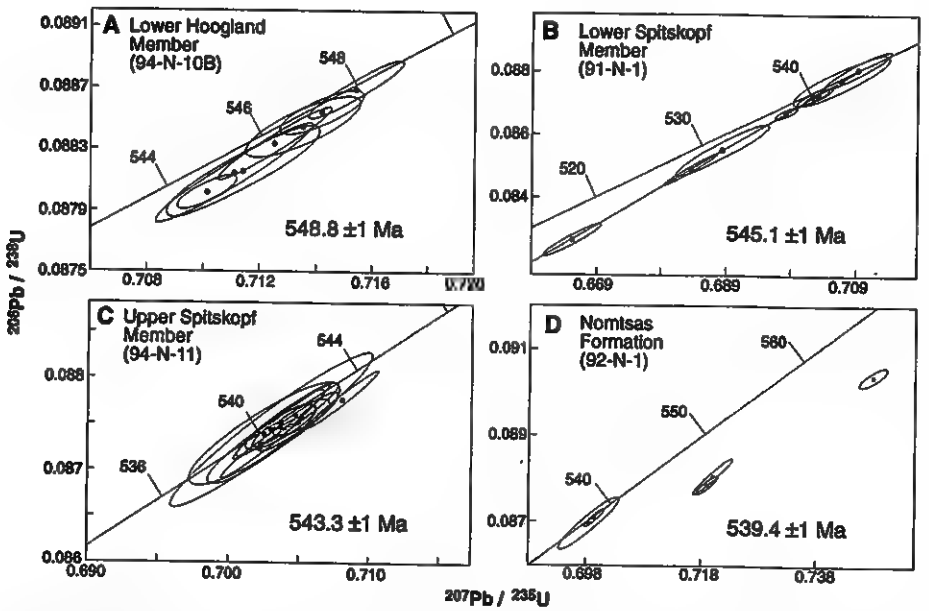

Fig. 3. Concordia diagrams for Nama ash beds dated in this study (**A** through **D**). Ages, in millions of years ago, are marked on the concordia curves. Individual analyses are depicted as 2σ error ellipses. See text for discussion.

U-Pb geochronology of Nama Group

ash beds. We separated zircons using standard techniques from the four samples of volcanic ash collected from the Nama Group. All zircons were air abraded (*56*) prior to dissolution. Both multigrain and single grain analyses (*13*, *57*) (Table 1) were performed for the samples, of which two show evidence of an inherited or detrital component.

Sample 94-N-10B [lower Hoogland Member (Fig. 1)] contains abundant clear, doubly terminated zircon crystals. Seven fractions of zircon, including four single grains, yielded a normally discordant ($^{207}Pb/^{206}Pb$ age > $^{207}Pb/^{235}U$ age > $^{206}Pb/^{238}U$ age) cluster of data that do not yield a satisfactory linear regression (Fig. 3A). The age of the sample is best estimated by use of the weighted mean of the $^{207}Pb/^{206}Pb$ ages calculated for all seven fractions. This mean $^{207}Pb/^{206}Pb$ age (95 percent confidence limit) is 548.8 ± 0.3 Ma (MSWD = 0.08) and we adopt an age of 548.8 ± 1 Ma as the best estimate of the age of this ash bed. Because this ash occurs below the base of the late Vendian +2 to +1 per mil carbon-isotopic interval that likely includes many of the world's most diverse assemblages of Ediacaran fossils (*18*, *21*, *22*, *25*), it represents a lower limit for the age of those fossils.

Sample 91-N-1 [lower Spitskopf Member (Fig. 1)] contains abundant, small (50–100 μm), euhedral, zircon grains, many of which are rich in inclusions. The zircons are normally discordant and define a linear array anchored by two concordant analyses (a and b in Table 1). Eight fractions, including four single grains, define a linear array with individual points ranging from 0 to 6.3% discordant. Regression of these eight analyses yields an upper intercept age of 545.6 + 2.5/− 1.9 Ma, a lower intercept of 22.3 ± 83.2 Ma with a MSWD of 0.2 (Fig. 3B). The upper intercept age of 545.6 Ma is interpreted to be the crystallization age of the zircons, but because the lower intercept is essentially 0, the weighted mean of the $^{207}Pb/^{206}Pb$ ages may be a more robust estimate of the crystallization age at 545.1 ± 0.7 Ma (MSWD = 0.22). We suggest that an age 545.1 ± 1 Ma is the best estimate of the age of sample 91-N-1, consistent with its stratigraphically higher position.

Sample 94-N-11 [upper Spitskopf Member (Fig. 1)] contains abundant clear zircons that range from elongate, needle-like zircons (100 to 150 μm in long dimension) to stubby, doubly terminated crystals (50 to 100 μm in long dimension). Fourteen fractions of zircon were analyzed, each containing from 2 to 13 grains (Fig. 3C). Most of the data define a tight cluster around concordia and thus preclude fitting of a regression line to the data. Three analyses (a, b, and c in Table 1) contain a small component of either inherited core material or older detrital grains. The age of the sample is best estimated from the weighted mean of the $^{207}Pb/^{206}Pb$ ages of the remaining 10

Table 1. Uranium-lead isotopic data. Zircon fractions are indicated by letters, and the number of grains is shown in parentheses. Sample weights, estimated using a video monitor with a gridded screen, are known to within 50% error.

Fraction	Weight (μg)	U (ppm)	Pb (ppm)	Total common Pb (pg)	Atomic ratios								Ages		
					$^{206}Pb^*/^{204}Pb$	$^{208}Pb\dagger/^{206}Pb$	$^{206}Pb\dagger/^{238}U$	%err	$^{207}Pb\dagger/^{235}U$	%err	$^{207}Pb\dagger/^{206}Pb$	%err	$^{206}Pb/^{238}U$	$^{207}Pb/^{235}U$	$^{207}Pb/^{206}Pb$
Lower Hoogland Member (94-N-10B)															
a(1)	4	1094.2	102.7	13.6	1668	0.140	0.08854	0.16	0.71419	0.21	0.05850	0.13	546.9	547.2	548.7
b(4)	4	322.0	29.5	3.0	2164	0.144	0.08850	0.45	0.71383	0.47	0.05850	0.10	546.6	547.0	548.7
c(1)	10	287.5	27.6	15.2	1017	0.145	0.08845	0.22	0.71354	0.29	0.05851	0.17	546.3	546.8	549.0
d(1)	5	472.9	43.5	3.9	3262	0.151	0.08829	0.27	0.71216	0.29	0.05850	0.07	545.4	546.0	548.5
e(5)	2	1039.4	99.4	9.4	1068	0.158	0.08816	0.34	0.71142	0.38	0.05853	0.16	544.6	545.6	549.6
f(3)	2	733.0	68.9	6.8	1340	0.154	0.08815	0.37	0.71111	0.39	0.05851	0.11	544.6	545.4	548.9
g(1)	4	1368.0	130.1	8.7	3701	0.185	0.08802	0.12	0.71013	0.15	0.05852	0.08	543.8	544.8	549.1
Lower Spitskopf Member (91-N-1)															
a(5)	3	264.7	25.6	7.5	667	0.158	0.08810	0.68	0.70923	0.73	0.05839	0.25	544.3	544.3	544.3
b(5)	6	98.6	9.6	6.8	530	0.161	0.08778	0.95	0.70675	1.07	0.05839	0.46	542.4	542.8	544.4
c(6)	9	246.3	23.6	11.4	1073	0.172	0.08726	0.28	0.70317	0.30	0.05844	0.11	539.3	540.7	546.4
d(1)	8	250.3	23.7	12.0	909	0.143	0.08723	0.32	0.70239	0.35	0.05840	0.14	539.1	540.2	544.8
e(17)	4	770.1	73.1	10.2	1582	0.183	0.08671	0.22	0.69822	0.26	0.05840	0.14	536.1	537.7	544.8
f(1)	3	195.4	18.0	6.6	531	0.127	0.08552	0.97	0.68845	1.05	0.05839	0.37	529.0	531.9	544.4
g(1)	7	140.9	12.7	3.4	1643	0.178	0.08492	0.59	0.68369	0.61	0.05839	0.12	525.4	529.0	544.6
h(1)	8	130.0	11.6	6.6	797	0.151	0.08261	0.64	0.66516	0.67	0.05840	0.20	511.7	517.8	544.6
Upper Spitskopf Member (94-N-11)															
a(10)	9	247.0	24.3	4.7	2895	0.166	0.09281	0.25	0.78297	0.29	0.06118	0.14	572.1	587.2	645.7
b(13)	6	241.4	24.0	14.6	558	0.164	0.08694	0.45	0.71322	0.75	0.05950	0.57	537.4	546.7	585.4
c(7)	8	188.6	17.4	3.4	2460	0.158	0.08776	0.35	0.70808	0.36	0.05851	0.08	542.3	543.6	549.1
d(12)	12	235.7	21.6	5.5	2942	0.150	0.08757	0.23	0.70520	0.28	0.05841	0.16	541.1	541.9	545.1
e(8)	7	233.5	22.1	5.2	1847	0.183	0.08752	0.37	0.70493	0.40	0.05842	0.14	540.8	541.7	545.5
f(2)	4	243.6	22.2	2.4	2288	0.147	0.08760	0.40	0.70476	0.43	0.05835	0.14	541.3	541.6	543.0
g(5)	5	267.1	25.0	5.0	1518	0.171	0.08754	0.45	0.70386	0.46	0.05832	0.11	540.9	541.1	541.7
h(10)	15	147.6	13.7	9.2	1346	0.139	0.08746	0.29	0.70371	0.36	0.05835	0.20	540.5	541.0	543.1
i(6)	7	171.0	15.6	3.2	2099	0.150	0.08750	0.44	0.70357	0.45	0.05832	0.12	540.7	540.9	541.7
j(6)	13	46.8	4.6	7.2	488	0.174	0.08744	0.97	0.70309	1.02	0.05832	0.29	540.4	540.6	541.7
k(4)	7	129.1	12.2	6.6	806	0.151	0.08739	0.64	0.70254	0.76	0.05831	0.38	540.1	540.3	541.3
l(8)	7	191.3	17.3	3.3	2342	0.141	0.08724	0.38	0.70223	0.39	0.05838	0.08	539.2	540.1	544.1
m(2)	3	271.1	25.1	3.0	1763	0.170	0.08726	0.47	0.70219	0.52	0.05836	0.22	539.3	540.1	543.4
Nomtsas Formation (92-N-1)															
a(18)	18	206.2	20.0	10.3	2015	0.167	0.09035	0.24	0.74780	0.33	0.06003	0.22	557.6	566.9	604.5
b(41)	10	318.9	30.2	8.1	2157	0.169	0.08805	0.46	0.72012	0.47	0.05931	0.12	544.0	550.7	578.7
c(11)	9	278.2	26.4	5.3	2715	0.187	0.08785	0.24	0.71906	0.26	0.05936	0.11	542.8	550.1	580.3
d(18)	18	174.1	16.4	8.4	2060	0.177	0.08708	0.22	0.69946	0.24	0.05826	0.09	538.2	538.5	539.4
e(6)	6	137.0	13.3	8.9	541	0.157	0.08696	0.69	0.69851	0.75	0.05826	0.26	537.5	537.9	539.4

*Measured ratio corrected for fractination only; Pb fractionation correction is 0.15% ± 0.03% per atomic mass unit. †Corrected for fractionation, spike, blank, and initial common Pb; U blank = 1 pg ± 50%; Pb blank = 3.5 pg ± 50%. Initial common Pb composition is calculated from Stacey and Kramer (*75*) with the interpreted age of the sample. Errors are reported in percent at the two-sigma confidence interval.

fractions. The mean $^{207}Pb/^{206}Pb$ age (95 percent confidence limit) is 543.3 ± 1.0 (MSWD = 0.86). The simplest interpretation of the data is that it is a slightly discordant array, and the best estimate of the age of the ash bed is 543.3 ± 1 Ma. This is interpreted as a maximum age for the Precambrian-Cambrian boundary.

Sample 92-N-1 [basal Nomtsas Formation (Fig. 1)] yielded few zircons that ranged from short stubby clear grains to cloudy inclusion-rich grains. Two single-grain analyses are concordant (d and e in Table 1), one of which has a rather large error ellipse because of a high common Pb content (Fig. 3D). The weighted mean of the $^{207}Pb/^{206}Pb$ ages for these points is 539.4 ± 0.3 Ma. Three additional analyses indicate the presence of a detrital or inherited component and have Pb-Pb ages that range from 577.6 to 603.6 Ma. We interpret 539.4 ± 1 Ma to be the best estimate of the age of this sample and a minimum age for the Precambrian-Cambrian boundary in Namibia.

Age of the Precambrian-Cambrian boundary and duration of Ediacaran animals. The age data of this study yield stratigraphically consistent results for the four ashes distributed over about 1000 m of section in the Nama Group. We have constrained the Precambrian-Cambrian boundary in Namibia to be younger than 543.3 ± 1 Ma and older than 539.4 ± 1 Ma, in good agreement with the upper intercept age of 543.8 + 5.1/−1.3 Ma (weighted mean 207/206 age of 543.9 ± 0.2 Ma) made on the basis of dating the lowermost Cambrian in Siberia (*13*).

A slight contradiction might be implied by the Siberian age of 543.9 Ma, which overlies the terminal Vendian negative $\delta^{13}C$ isotopic excursion, and the Namibian age of 543.3 Ma, which is inferred to underly the same excursion, as we have assumed that the excursion in both localities is related to global seawater composition. However, the analytical uncertainties for the two age determinations overlap, and we take this to indicate that the negative carbon-isotopic excursion lasted 1 million years or less. Furthermore, the surfaces interpreted to include the Precambrian-Cambrian boundary in two important reference sections have approximately the same age, despite strong paleogeographic separation. Whereas the Nama Group was deposited in a foreland basin, isolated near the center of the Gondwanan supercontinent, Siberia wandered independently among a separate collage of continental fragments (*58*, *59*).

Our results indicate that the Ediacaran metazoans had a substantial age range with a much younger upper limit than previously thought. Recently, there have been suggestions that some Ediacaran fossils may have a younger upper age limit than was conventionally accepted (*30*, *60*, *61*). By having calibrated in absolute time an important tie point in the carbon isotope record (*62*) we arrive at the general conclusion that the most diverse Ediacaran fossil assemblages are no more than about 6 million years older than the Precambrian-Cambrian boundary (Fig. 4). Furthermore, the *Pteridinium* and other specimens near the top of the Spitskopf Member provide direct evidence, confirmed by the 543 Ma age for the upper Spitskopf ash, that Ediacaran organisms existed essentially up to at least the time of the Precambrian-Cambrian boundary.

The minimum lower limit for the age range of the Ediacaran fauna is 565 Ma as constrained at Mistaken Point, Newfoundland (*29*). Including this age, we estimate that Ediacaran organisms existed for at least 20 million years. This range may be broadened considerably if the fossils of the Twitya Formation, northwestern Canada, are included. These disk and ring-shaped impressions constitute a low-diversity assemblage of forms that are akin to the simplest representatives from the Ediacaran fauna of the Russian platform (*17*, *21*). An approximate age for the Twitya fossils is provided because they underlie Varanger age glaciogenic rocks estimated to have formed close to 600 Ma, as discussed above. Consequently, tissue-grade multicellularity probably evolved at least 35 million years before the oldest dated diverse Ediacaran faunas. Considered collectively, the record of macroscopic animal evolution is interpreted to span a minimum of 55 million years before the Precambrian-Cambrian boundary (Fig. 4).

Implications for early animal evolution. A large gap in the record has been long perceived to exist between the youngest Ediacaran fossils and the oldest diverse invertebrate fossil assemblages near the base of the Cambrian System. However, it now seems likely that the Ediacaran animals existed throughout much of the Vendian (Fig. 4) and that early faunal groups may have experienced greater overlap than previously recognized; the lesson learned from the fossils at the top of the Spitskopf is a reminder that their absence in other contemporaneous strata is more likely an artifact of preservation than an evolutionary obituary. At the same time, a growing number of skeletalized invertebrate fossils can be shown to overlap with the Ediacaran fossils, extending well below the Precambrian-Cambrian boundary. *Cloudina*, once thought to be the only Vendian skeletalized invertebrate, is now joined by the goblet-shaped fossils of the Nama Group. Their ranges completely overlap with the most diverse Ediacaran fossil assemblages (Fig. 4), and they are locally so abundant that they form bioclastic sheets. In addition, through correlation of carbon isotope anomalies, some Cambrian-aspect shelly fossils may now have ranges that extend into the Vendian. *Anabarites* and *Cambrotubulus* appear in uppermost (negative $\delta^{13}C$ values) Vendian strata of Siberia (*53*, *63*, *64*), and *Anabarites* may be present in somewhat older (positive $\delta^{13}C$ values) strata of Mongolia (*65*).

Once held as the position in the rock record where the major invertebrate groups

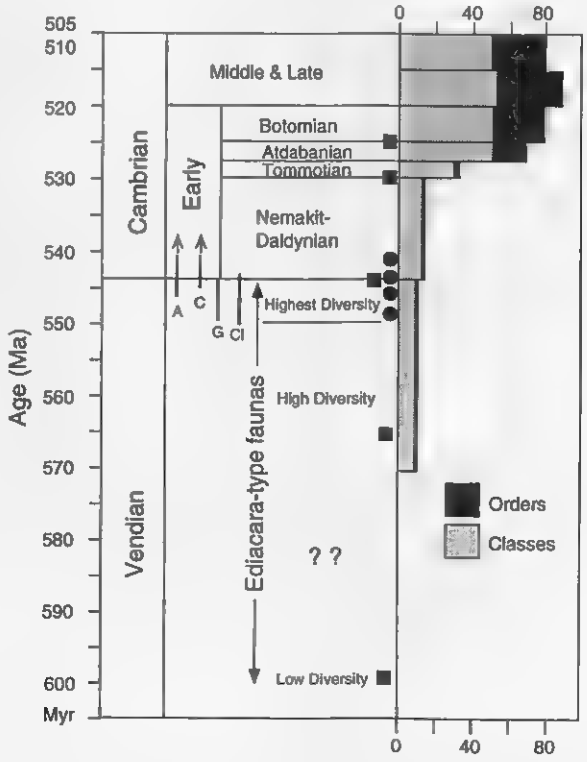

Fig. 4. Revised time scale for the Vendian and Cambrian periods. Key boundaries and events that are constrained by U-Pb zircon chronology are marked by filled circles (this article) or filled squares [other data referenced in the text and in (*13*)]. Diversity data is from Sepkowski (*66*) and diversity divisions for Ediacaran taxa are from Narbonne *et al.* (*21*). Ranges of Vendian age skeletalized fossils are shown: A, *Anabarites*; C, *Cambrotubulus*; Cl, *Cloudina*; and G, goblet-shaped fossils in Nama Group.

first appeared, the Precambrian-Cambrian boundary now serves more as a convenient reference point within an evolutionary continuum. Skeletalized organisms, including Cambrian-aspect shelly fossils, first appear below the boundary (*46*, *64*, *65*) and then show strong diversification during the Early Cambrian (Fig. 4) (*8*, *66*–*68*). Similarly, trace fossils also appear first in the Vendian, exhibit a progression to more complex geometries across the boundary, and then parallel the dramatic radiation displayed by body fossils (*23*, *24*).

Perhaps the Precambrian-Cambrian boundary will acquire new significance in marking the point of extinction of some or all of the Ediacaran organisms given that the impressions at the top of the Spitskopf Member occur just below the boundary. Such a record might be a predictable outcome of extensive predation by more advanced Cambrian organisms (*11*, *69*), although this interpretation conflicts with our evidence that the Ediacaran organisms probably existed concurrently with other major invertebrate groups, including macrophagous predators (*70*). Alternatively, by filling in most of the temporal gap between Ediacaran and Cambrian faunas, the Ediacaran fossils at the top of the Spitskopf Member in Namibia could be used to support evolutionary models that interpret the Ediacaran organisms as ancestors to certain Cambrian metazoans (*9*, *71*–*73*), or as a sister group to the metazoans (*74*). Our data do not force abandonment of any of these hypotheses. Considered collectively, however, the most parsimonious interpretation of the available fossil and age data is that the early development of animals proceeded as a single, protracted evolutionary radiation, culminating in the Cambrian explosion (Fig. 4).

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Nano-Elastohydrodynamics: Structure, Dynamics, and Flow in Nonuniform Lubricated Junctions

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Structure, flow, and response characteristics of molecularly thin films of hexadecane, sheared by topographically nonuniform solid gold surfaces sliding at a relative velocity of 10 meters per second, were investigated with molecular dynamics simulations. The simulations reveal three characteristics: spatial and temporal variations in the density and pressure of the lubricant in the region confined by the approaching asperities, accompanied by asperity-induced molecular layering transitions that are reflected in oscillatory patterns in the friction force; asperity deformations and microstructural transformations mediated by the lubricant; and an onset of cavitated zones in the lubricant after the asperity-asperity collision process. The simulations extend micrometer-scale elastohydrodynamic investigations into the nanometer-scale regime and provide molecular-scale insights into the fundamental mechanisms of ultrathin film lubrication phenomena under extreme conditions.

The general purposes of a lubricating fluid are to provide a protective coating to the solid surfaces, thus preventing formation of adhesive junctions, and to reduce frictional energy losses by acting as an interfacial layer of low shear strength (*1*–*3*). Underlying the development in the early 1960s of the theory of elastohydrodynamic lubrication (EHL) (*3*, *4*) was the observation that when two nonconforming solid surfaces come into contact in the presence of a liquid lubricant (or when two asperities of nominally conforming surfaces, under conditions of boundary lubrication, come together), the pressure developed in the contact zone may achieve such high values that local elastic deformations of the surfaces must be included in proper treatments of lubrication of the tribosystem. The subsequent development of micrometer-scale EHL (micro-EHL) and numerical algorithms (*3*, *4*) allowed investigators to focus on lubrication processes involving individual surface irregularities (asperities) and has aided the design of machine elements and bio-tribological systems (*5*, *6*) of improved efficiency and durability. Inherent to these continuum models are certain assumptions that are used as input into the calculations. These include constitutive relations such as rheological properties of the lubricant film (Newtonian or non-Newtonian viscosity laws, as well as pressure and temperature variations of the viscosity) and mechanical response characteristics of the bounding surface materials (substrates and asperities), as well as imposed interfacial liquid-solid boundary conditions.

The ever-increasing trend toward miniaturization of technological devices and the ability to fabricate such structures, coupled with the emergence and proliferation of proximal probes (in particular, tip-based microscopies and the surface force apparatus) and of atomic-scale simulation techniques, provide the impetus and the means for nanometer-scale modifications and manipulations of materials and allow systematic investigations of interfacial problems of fundamental and technological interest with unprecedented high spatial and temporal resolutions and under extreme conditions (*7*, *8*). Through such investigations it was found that molecular structure and dynamics, mechanical response, and rheology at interfaces and in confined thin films are often very different from those in the bulk and cannot be understood by simple extrapolation of bulk properties (*7*). However, even though the surfaces of even the most highly polished engineering components are characterized by roughness on a broad range of length scales (*9*), most recent work, using atomic-force and friction-force microscopies, the surface force apparatus (SFA), and molecular dynamics (MD) simulations, focused on atomically structured flat and smooth confining surfaces (*7*).

Asperity-asperity collisions, formation and subsequent breakage of interfacial adhesive junctions, and shear-induced rheological transformations in highly confined fluids are among the most fundamental processes in tribology as well as boundary and thin-film lubrication (*1*, *7*). To investigate the atomic and molecular origins of such processes in morphologically nonuniform lubricated narrow junctions sheared at high velocities we have used MD simulations (*7*, *8*), thus extending micro-EHL investigations to the nanometer-scale regime, is beyond the range of applicability of continuum-based models. In such simulations the equations of motion of a system of particles interacting by means of prescribed interatomic potentials are numerically integrated, and the resulting phase-space trajectories are analyzed. For model systems we used thin films of hexadecane (n-$C_{16}H_{34}$) confined between two gold substrates exposing (111) surfaces [that is, Au(111)]. Topographical nonuniformities (asperities) were modeled by flat-top pyramidal gold structures of height h_a from the underlying gold surface, extending a finite length in the x direction and over the whole simulation cell in the y direction (that is, asperity ridges; see views along the y direction in Fig. 1).

The interactions between the gold atoms were modeled by many-body embedded-atom method interactions (*10*), which provide a proper description of the nature of cohesion in metals. The alkane molecules were simulated by using the united atom model (*11*, *12*), with the interaction potentials between the CH_2 and CH_3 segments of the molecules including harmonic intramolecular bond-stretch and bond-angle bending potentials, a dihedral angle potential, and nonbonded intra- and intermolecular interactions. The interactions between the molecular segments and the gold atoms were modeled by 6–12 Lennard-Jones potentials with parameters fitted to experimentally estimated desorption data (*12*). These potentials have been tested and used successfully in previous studies of bulk and interfacial alkane systems (*11*–*13*).

The Newtonian equations of motion were integrated by using the Verlet algorithm (*14*), with an integration time step $\Delta t = 3.06 \times 10^{-15}$ s; for each of our systems a typical simulation consisted of 4×10^5 Δt, that is, 1.2 ns, past a prolonged equilibration stage. In simulations of shear-induced flow, the solid surfaces were kept at a constant separation (chosen to correspond to an initially vanishing average normal load) and were translated along the x axis in opposite directions, with the top and bottom surfaces moving with a constant velocity of +5 and −5 m/s (a relative sliding velocity $V = 10$ m/s). In these simulations the alkane molecules and the gold atoms of the asperities were treated dynamically. The simulations were performed isothermally, at a temperature $T = 350$ K, through scaling of intramolecular velocities in 50 Δt intervals.

To explore the dependence of structure, dynamics, and flow on the characteristics of the lubricated junctions, we studied mainly three lubricated systems. The first consists of 422 hexadecane molecules with the separation between the gold surfaces $S = 36.1$ Å, the height of each of the asperities $h_a = 9.3$ Å, and thus the separation between the asperity tops $\Delta h_{aa} = S - 2h_a = 17.5$ Å; the second system, which we refer to as the near-overlap case, consists of 243 molecules, with $S = 23.2$ Å, $h_a = 9.3$ Å, and $\Delta h_{aa} = 4.6$ Å; and the third system, referred

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to as the overlap case (see Fig. 1), consists of 201 molecules, with $S = 21.3$ Å, $h_a = 14$ Å, and $\Delta h_{aa} = -6.7$ Å. In describing our results we denote by d_{aa} the distance along the x direction between the leading edges of the opposing asperities. For each system the simulations of sliding followed equilibration, with the asperities well distanced from each other, such that steady-state flow could be established before the onset of effects caused by lubricant confinement in the interasperity region.

Atomic and molecular configurations of the three simulated systems, recorded in each case at selected times during the shearing process, are displayed in Fig. 1. The main patterns illustrated by these configurations are (i) the evolution of liquid layered structures in the region between the colliding asperities and (ii) the severe deformations of the asperities for the cases of near-overlapping (Fig. 1B) and overlapping (Fig. 1C) asperity heights. It is particularly noteworthy that although in all cases a certain degree of interfacial layering in the vicinity of the solid boundaries already existed at equilibrium and for large transverse separations between the asperities, the layering of the lubricant in the interasperity region was initially absent and developed dynamically as the asperities approached each other. Furthermore, the number of layers in the interasperity region evolved in each case in a "quantized" manner as successive layers were squeezed out of it when the asperities came closer together.

These atomic and molecular structural variations are portrayed in the behavior of the forces acting between the thin-film molecules and the solid substrates, as well as by the intermetallic interactions between the two solid surfaces. As a demonstration we show in Fig. 2 the time variation of the forces in the shear and normal directions (f_x and f_z, respectively) recorded during a simulation of the near-overlapping system. Of particular interest are the oscillatory patterns in the forces before the collision between the asperities, whose characteristics correlate with the structural layering stages of the lubricating film (compare Figs. 1B and 2; note that the marked minima in f_x and f_z, seen particularly clearly in f_x, correspond to successively decreasing discrete numbers of molecular layers in the interasperity region).

The aspects revealed by our investigations are that molecular density layering and consequent solvation force oscillations can occur not only in liquids confined between smooth solid surfaces under equilibrium conditions (*7*), but also under constant-velocity shear-flow conditions in a lubricated junction with nonuniform surfaces, and that such oscillations, correlated with quantization of the number of lubricant layers in a localized region of the contact (that is, the interasperity zone), can be exhibited both in the normal (f_z) as well as the tangential (f_x) forces. The implication of these observations (which we have made also for the two other simulated systems) is that such oscillations in the force resisting the relative sliding motion (that is, the friction force, f_x) may lead to stick-slip behavior in experiments where the sliding of the substrates (kept at constant normal separation) is driven by means of a connection through a spring element to a stage dragged at constant velocity [as in SFA measurements (*7*, *15*)].

Another aspect revealed by the simulations pertains to the crucial importance of the dynamical mechanical response of the substrates, and in particular that of the irregularities (asperities), in determining the evolution and properties of sheared systems, as is illustrated through the atomic configurations shown in Fig. 1 and the force and local stress plots shown for the near-overlap

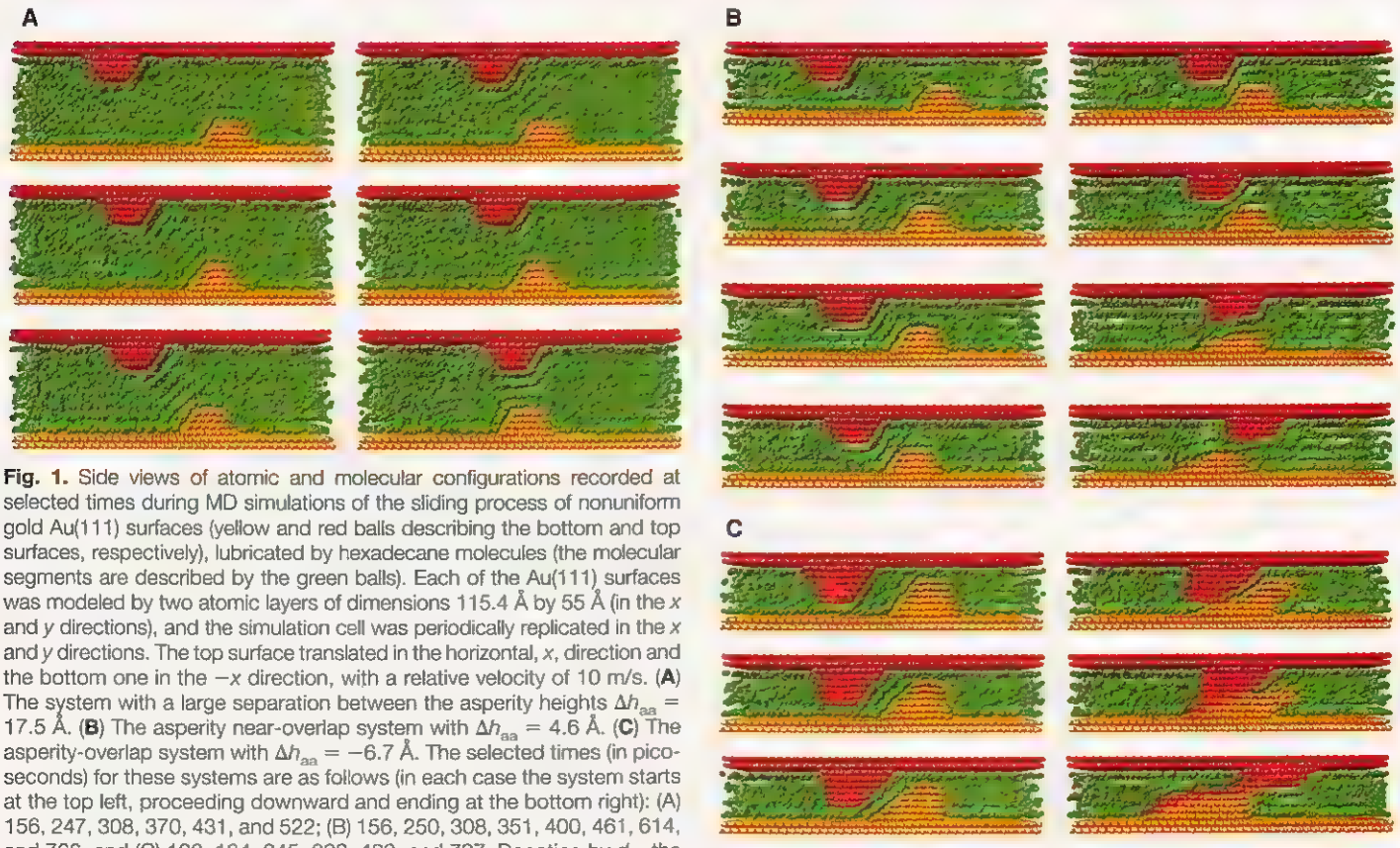

Fig. 1. Side views of atomic and molecular configurations recorded at selected times during MD simulations of the sliding process of nonuniform gold Au(111) surfaces (yellow and red balls describing the bottom and top surfaces, respectively), lubricated by hexadecane molecules (the molecular segments are described by the green balls). Each of the Au(111) surfaces was modeled by two atomic layers of dimensions 115.4 Å by 55 Å (in the x and y directions), and the simulation cell was periodically replicated in the x and y directions. The top surface translated in the horizontal, x, direction and the bottom one in the $-x$ direction, with a relative velocity of 10 m/s. (**A**) The system with a large separation between the asperity heights $\Delta h_{aa} = 17.5$ Å. (**B**) The asperity near-overlap system with $\Delta h_{aa} = 4.6$ Å. (**C**) The asperity-overlap system with $\Delta h_{aa} = -6.7$ Å. The selected times (in picoseconds) for these systems are as follows (in each case the system starts at the top left, proceeding downward and ending at the bottom right): (A) 156, 247, 308, 370, 431, and 522; (B) 156, 250, 308, 351, 400, 461, 614, and 766; and (C) 122, 184, 245, 398, 489, and 797. Denoting by d_{aa} the distance along the x direction between the leading edges of the approaching asperities, conversion of the above times to d_{aa} distances can be achieved by $d_{aa} = d_0 - 0.1t$, where $d_0 = 38.7$ Å [for (A) and (B)], $d_0 = 18.7$ Å [for (C)], and t is in picoseconds. Note the layering in the interasperity zone for all three systems and asperity deformations in (B) and (C).

system in Figs. 2 and 3, respectively. For relatively large spacing between the asperity heights (Δh_{aa} = 17.5 Å, Fig. 1A), shear-flow accompanied by structuring of the lubricant occurs with no distortions of the metal surfaces. However, in the cases of nearly overlapping (Fig. 1B) and overlapping (Fig. 1C) surface irregularities, large densification and pressurization of the lubricant in the interasperity region occur, accompanied by a significant increase of the effective viscosity in that region [detailed analysis of the rheological and dynamical characteristics indicate formation of a viscoelastic or elastoplastic zone (*16*)]. These processes result in deformations of the gold asperities, much beyond the elastic response regime.

For the case of near asperity overlap (Figs. 1B, 2, and 3), the gradually increasing confinement of the interasperity region is accompanied by the development of transient local stresses in front of the moving asperities (in Fig. 3 local stresses normal to the interfaces, τ_{zz}, and along the shear direction, τ_{xz}, are shown). Similar results were obtained for the overlapping-asperity case with even larger magnitudes of the stresses. The local shear and normal stresses that develop in the junction are of comparable magnitudes, with $\tau_{zz} > \tau_{xz}$ when the asperities come closer together (see t = 585 ps in Fig. 3). On exceeding a limiting value of close to 4 GPa, these accumulated local stresses lead to severe deformations and structural transformations of the asperities (*17*), mediated by the intervening lubricant molecules, even before the onset of direct intermetallic interactions (Figs. 1B, 2, and 3). In some locations along the asperity ridge (in the y direction), drainage of the lubricant molecules was complete when the sliding asperities passed over each other, leading to the formation of an intermetallic connective junction that sheared and eventually broke with continued sliding, resulting in the transfer of some metal atoms between the asperities. In this context we remark that in comparative simulations under the same conditions with no lubricant molecules in the junction, the magnitude of the interaction between the asperities and the degree of deformation were found to be insignificant. Furthermore, when the shearing process of the lubricated system was simulated with undeformable asperities, normal and tangential stresses in excess of 150 GPa developed, accompanied by trapping of a monolayer of alkane molecules between the asperities. This observation emphasizes that the dynamical mechanical response of the surfaces, and in particular, that of the nonuniform surface features (asperities), must be included for a proper description of the lubricated junction under these conditions.

Asperity deformations, complete lubricant drainage, growth of intermetallic junctions, shear-induced metal epitaxy, partial spreading by means of interlayer slip in the intermetallic junctions resulting in structural modifications of the surface topography (smoothing), and eventual rupture of such junctions are most pronounced for the case of overlapping asperities (Fig. 1C). The results for both this system and the near-overlap one suggest that in addition to the aforementioned lubricant-mediated smoothing of surface roughness, oscillatory shear or cyclic relative motion of the surfaces may lead to material fatigue, wear, and eventual failure due to repeated stress loading cycles of the surfaces.

For the near-overlap system, we comment also on the occurrence of local rupture, after asperities have collided, of the liquid film in the region between the departing asperities, resulting in the appearance of a nanometer-scale cavitated zone (*18*) (of a length scale of ~30 Å and lasting for a period of more than 100 ps). The appearance of such cavitated zones is associated with insufficient back-flow of lubricant molecules into that region. The formation and consequences of cavitated zones in lubricating films, which are subjects of basic and technological significance, have been recently discussed in the context of micro-EHL simulations (*19*) of film breakdown as two rough surfaces slide against each other.

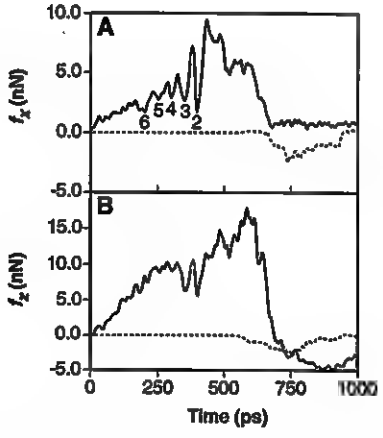

Fig. 2. (**A** and **B**) Total forces (in the x direction, f_x, and in the direction normal to the surfaces, f_z) on the gold solid surfaces from the alkane molecules (solid lines) plotted versus time for the near-overlap system (see Fig. 1B; for conversion to interasperity distances, see caption). Intermetallic forces between the opposing solid gold surfaces, with an onset on close approach of the asperities, are depicted by the dashed line. The numbers in f_x designate the "quantized" number of layers in the interasperity zone (see Fig. 1B). Note the correlation between the force oscillations and the structural variations in the lubricant.

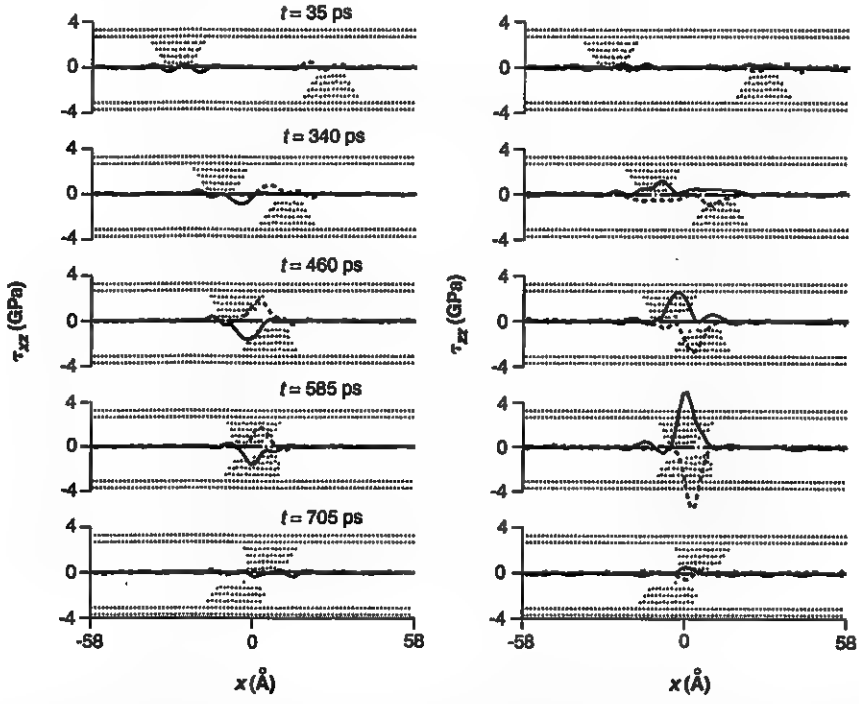

Fig. 3. Local stresses, exerted by the alkane molecules on the solid gold nonuniform substrates, plotted versus x. The stresses, obtained during a sliding simulation of the near-overlap system (see Fig. 1B), were calculated by averaging forces across the y direction. The solid and dashed lines denote the stresses on the solid top and bottom surfaces, respectively. Side views of the solid surfaces are superimposed, aiding in visualizing the relative interasperity configuration at the indicated times. Note the significant local stresses developing in front of the approaching asperities and the asperity deformations when the stresses achieve values of ~4 GPa.

Our findings provide insights into the atomic-scale fundamental processes of ultra-thin film lubrication, thus extending continuum EHL and micro-EHL treatments into the nanometer realm. Such investigations address some issues facing certain current novel technologies [such as high-density information storage and retrieval systems (*20*) and provide the impetus for future experimental and theoretical investigations.

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Imaging Pattern Formation in Surface Reactions from Ultrahigh Vacuum up to Atmospheric Pressures

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Two optical methods that allow pattern formation to be investigated at an arbitrary pressure are here applied to image concentration patterns of adsorbed species associated with heterogeneous catalytic reactions. In contrast to most surface physical techniques, these methods are not restricted to high vacuum conditions and thus bridge the "pressure gap." With carbon monoxide oxidation on a (110) surface of platinum as an example, the coupling mechanisms responsible for spatiotemporal self-organization in surface reactions were followed from reaction-diffusion control to the thermokinetic region, associated with phenomena not previously observed in pattern formation.

The applicability of most surface physical techniques is restricted to ambient gas pressures below about 10^{-3} mbar. This gives rise to a "pressure gap" between model studies conducted at low pressures and the conditions of "real" catalysis. Data measured at low pressures exploring the mechanism and kinetics of a heterogeneously catalyzed reaction can be theoretically extrapolated across a large pressure range (*1*); however, there are also cases in which the phenomena occurring on opposite sides of this gap are different in nature. This is the case for nonlinear effects causing spatiotemporal pattern formation on a reacting surface (*2*): At low pressures the conditions are nearly isothermal, and concentration patterns are formed by coupling of the reaction and surface diffusion. Phenomena of this type have been widely studied with the recently developed technique of photoemission electron microscopy (PEEM) (*3–5*). At higher pressures, on the other hand, finite temperature differences caused by variations of the heat release associated with the reaction give rise to thermokinetic waves governed by the heat conductance of the system, which to date have been imaged primarily by techniques based on infrared emission (*6*).

In this report we demonstrate how two optical methods may be used to image surface patterns at arbitrary pressure. With these techniques, the continuous range of coupling mechanisms responsible for spatiotemporal self-organization in surface reactions (for example, diffusion and heat conductance) becomes accessible to experimental investigation. The two techniques, ellipsomicroscopy for surface imaging (EMSI) and reflection anisotropy microscopy (RAM), are closely related and are based on changes of the degree of polarization of light reflected from a surface.

The EMSI technique is based on ellipsometry, which is well-established and has been used as an imaging method for characterizing structures on surfaces with thick-

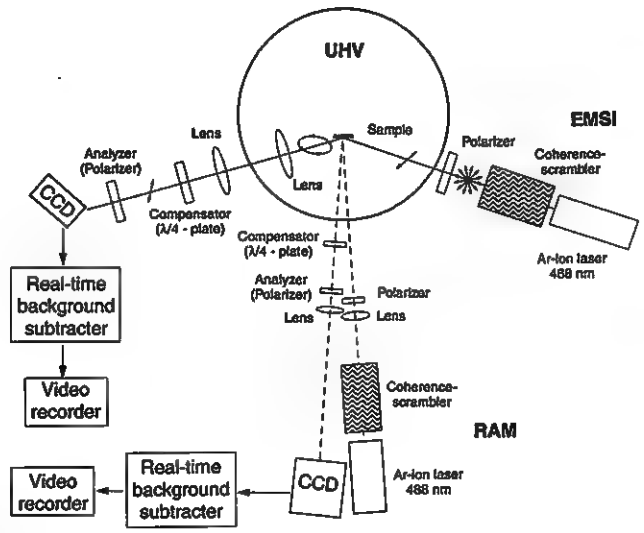

Fig. 1. Experimental setup for EMSI and RAM. Light from an Ar-ion laser (wavelength $\lambda = 488$ nm, power = 100 mW) is reduced in coherence as it passes through a vibrating multimode optical fiber; it is then linearly polarized and illuminates the surface at an angle of 70° with respect to its normal for EMSI (solid path) and close to normal incidence for RAM (dashed path). After reflection, the beam is imaged onto a charge-coupled device (CCD) chip by lenses (enabling magnifications between ×1 and ×50), after passing through a compensator ($\lambda/4$ plate) and being adjusted close to zero intensity by the analyzer. An image processor (Hamamatsu Argus 20) permits background subtraction in real time. Arrows indicate the polarization of the light for EMSI.

nesses greater than several nanometers (7). We developed the EMSI technique to allow real-time imaging of dynamic phenomena in distributions of submonolayer quantities of atoms adsorbed on a metal surface from ultrahigh vacuum (UHV) to atmospheric pressures. Although EMSI is presented here as a more qualitative tool, it should be possible to obtain quantitative information about the observed systems from measurements and corresponding calibration of the common ellipsometric parameters Δ and Ψ (8). Thus, in principle the concentrations of chemisorbed species on the surface could be inferred from the changes in Δ and Ψ relative to the clean surface; however, for practical purposes calibration of the brightness through well-defined adsorbed layers should usually be appropriate.

Sensitivity in surface imaging comparable to that with EMSI is achieved if the beam is reflected near normal incidence (Fig. 1). In this mode, the optical reflectivity along the two inequivalent directions of an anisotropic surface is probed; therefore, we denote this method as reflection anisotropy microscopy. It requires that the (azimuthal) polarization angle of the incident light be adjusted between the two principal axes of an anisotropic surface, such as the (110) plane of a face-centered-cubic crystal. This takes advantage of the fact that the anisotropy in reflectivity is often changed by the presence of a submonolayer coverage of adsorbates (9), caused by, for example, a surface reconstruction or the formation of an overlayer with a unit cell of different symmetry.

For both techniques, the reflected light is extinguished by appropriate settings of the compensator and analyzer, such that the image is dark for a uniform, featureless surface. For the EMSI image, local deviations of the ellipsometric parameters—that is, the complex refractive index and the thickness of surface layers—appear as brighter areas. For RAM, regions of different reflection anisotropy are contrasted. We conducted the experiments presented here in an UHV system using both methods independently, but EMSI and RAM can be applied simultaneously, thereby providing supplementary information.

We studied the catalytic oxidation of carbon monoxide on a Pt(110) surface, a system whose mechanism and dynamic properties have been explored in detail (4). The reaction proceeds through surface recombination of chemisorbed O and CO species (formed by adsorption of gaseous O_2 and CO), whereby the CO_2 produced by the reaction is immediately released into the gas phase. Under certain conditions of temperature T and partial pressures p_{O_2} and p_{CO}, the reaction rate becomes oscillatory or even chaotic, and the surface concentrations of adsorbed O and CO are not uniform, but rather exhibit spatiotemporal patterns between regions of high O coverage (with a high reactivity) and regions of high CO coverage (less reactive). These patterns have been studied in detail at partial pressures below 10^{-3} mbar with the PEEM technique (10).

In experiments at pressures below 10^{-3} mbar, where PEEM is applicable, EMSI and RAM revealed the same patterns observed with PEEM, but with a much-enlarged field of view, up to the full sample size. This allowed the observation of patterns on a larger length scale. Contrast was observed in RAM images (Fig. 2) as a result of differences in the anisotropy of surface reflectivity. This was verified by turning the azimuth of the sample during pattern formation. When the [001] or [1$\bar{1}$0] directions of the crystal were aligned with the plane of polarization of the incident light, contrast was lost. Dark and bright areas in the image can be attributed to domains predominantly covered by CO and O, respectively. This was verified by comparison with experiments in which the surface was uniformly covered either by CO or O.

Fig. 2. Pattern formation during the CO oxidation on Pt(110) for $p_{O_2} = 4 \times 10^{-4}$ mbar and $p_{CO} = 6.2 \times 10^{-5}$ mbar at $T = 494$ K, recorded with RAM, showing the development of a CO-rich target pattern surrounded by O-rich areas of the surface. Target patterns observed previously (under different conditions) with PEEM (10) exhibited wavelengths on the order of 10 μm; the wavelength here is about 1 mm.

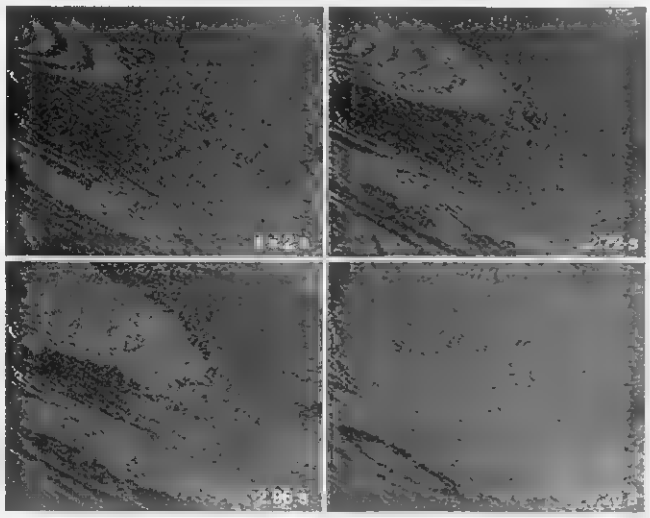

Fig. 3. Transition from the less reactive (CO-rich) into the highly reactive (O-rich) state, recorded by EMSI. At $t = 0$, p_{CO} was lowered from 0.07 to 0.06 mbar with $p_{O_2} = 0.5$ mbar and $T = 550$ K. At $t = 2$ s, an O-rich spiral wave appears and then propagates into the CO-covered region. Extinction was adjusted for the homogeneously CO-covered surface. Areas covered by O thus appear brighter. The image diameter is 1.4 mm.

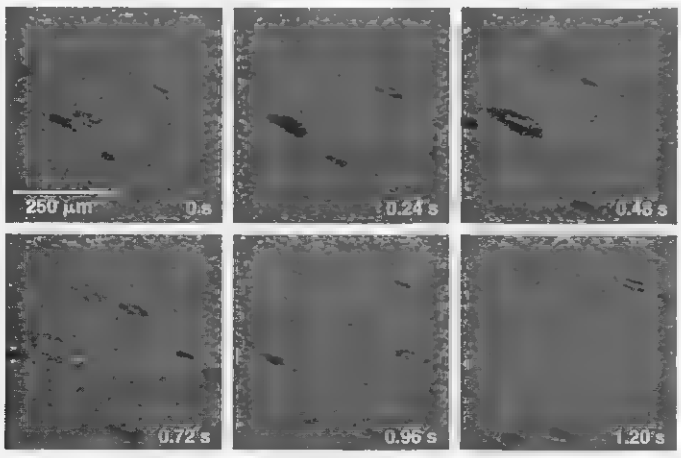

Fig. 4. Raindrop-like patterns at $p_{O_2} = 2.22 \times 10^{-2}$ mbar, $p_{CO} = 5 \times 10^{-3}$ mbar, and $T = 534$ K observed with EMSI.

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Under low-pressure conditions ($<10^{-3}$ mbar), the sample temperature remained constant, unaffected by variations of the surface composition and reactivity. Such patterns have previously been modeled theoretically by appropriate reaction-diffusion equations (*4*, *5*). However, when the pressure was increased by more than two orders of magnitude (into the pressure range not accessible by PEEM), the situation changed markedly. Because of the higher turnover rate, the sample temperature was considerably affected by the released heat of reaction. For the gas composition with the highest turnover, the increase in temperature was as high as 40 K at a total pressure of 0.5 mbar. When the CO/O_2 partial pressure ratio was further increased, the surface changed from the reactive into the less reactive state, associated with a rapid decrease of the sample temperature. To reestablish a high reaction rate, we had to intermediately lower p_{CO}. The resulting transient state is reflected in EMSI images (Fig. 3). At $t = 2$ s, an O-rich spiral wave appeared. This spiral propagated into the CO-covered region, such that after 3 s, roughly half of the imaged surface area was in the reactive O-rich state. Because of the increase of reactivity, the sample temperature rose, which further enhanced the decrease of the CO concentration by desorption, such that after about 3.2 s, the whole surface was in the predominantly O-covered and highly reactive state—that is, had turned bright. The speed of the reaction front was determined from the video frames between the last two images of Fig. 3 to be about 5 mm/s.

We have been able to observe pattern formation and propagating reaction fronts up to total pressures of 1 atm. Limitations at even higher pressures were encountered because the UHV chamber used in the experiments is unsuitable for such conditions.

A previously unseen type of pattern was found (Fig. 4) at $p_{O_2} = 2.22 \times 10^{-2}$ mbar. Its features are reminiscent of target patterns known from pure reaction-diffusion behavior at lower pressures (*4*). However, while the latter are periodically emitted from fixed trigger centers (presumably surface defects) and propagate continuously, the present patterns appear at random, like raindrops on a flat water surface rapidly dying out after a short propagation length. This behavior may be described by the superposition of reaction-diffusion and thermokinetic effects. The pronounced damping of wave propagation and the role of nonisothermal effects in this system will have to be analyzed in the future by detailed theoretical modeling.

The rather simple optical methods EMSI and RAM presented here allowed the investigation of pattern formation associated with heterogeneously catalyzed reactions from UHV up to atmospheric pressures. An upper pressure limit for the applicability of these methods is not apparent. In addition, with the spatial resolution principally limited by diffraction, these methods will enable the study of other surface processes occurring at length scales from the submicrometer up to several millimeters, and the present temporal resolution of 20 ms can certainly be improved significantly.

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Nanoscale Complexity of Phospholipid Monolayers Investigated by Near-Field Scanning Optical Microscopy

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Near-field scanning optical microscopy of phospholipid monolayers doped with fluorescent lipid analogs reveals previously undescribed features in various phases, including a concentration gradient at the liquid-expanded/liquid-condensed domain boundary and weblike structures in the solid-condensed phase. Presumably, the web structures are grain boundaries between crystalline solid lipid. These structures are strongly modulated by the addition of low concentrations of cholesterol and ganglioside G_{M1} in the monolayer.

Lipid monolayers have been used to study two-dimensional systems, to construct organized arrays and matrices, and to model biological membranes (*1*). An understanding of their structure is critical to all of these efforts. At the molecular level, much has been learned from x-ray and electron diffraction (*2*), scanning tunneling microscopy (STM), and atomic force microscopy (AFM) (*3*). It is also possible to analyze lipid monolayers on a larger scale by far-field epifluorescence microscopy (FFM) (*4*–*7*) of monolayers doped with fluorescent lipid analogs. Such studies have shown that, in the fluid-solid coexistence region, lipid monolayers exhibit domain structures. The size and shape of these domains vary with temperature, pressure, and the chemical composition of the monolayer. In particular, low concentrations of certain compounds (such as cholesterol) alter the line tension at the boundary between two coexisting phases, thereby changing the domain

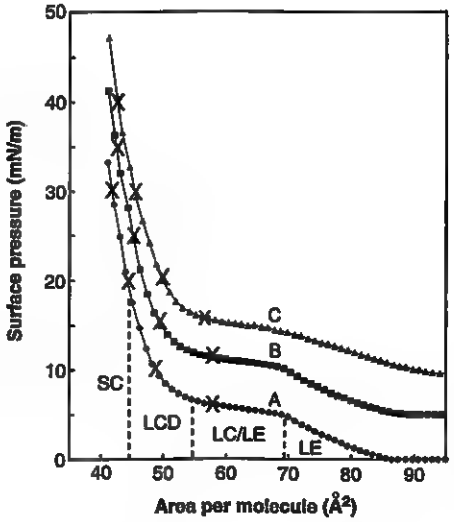

Fig. 1. The πA isotherms of (curve A) DPPC/0.5 mol % Bodipy-PC, (curve B) DPPC/0.5 mol % Bodipy-PC/1 mol % cholesterol, and (curve C) DPPC/0.5 mol % Bodipy-PC/0.5 mol % ganglioside G_{M1} monolayers at the air-water interface. For clarity, isotherms B and C are shifted upward with an offset of +5 and +10 mN/m, respectively, in surface pressure. Four different regions (LE, LE/LC, LCD, and SC) corresponding to different lipid phases are distinguished. Points at which the monolayers were sampled are marked by X's on the curves.

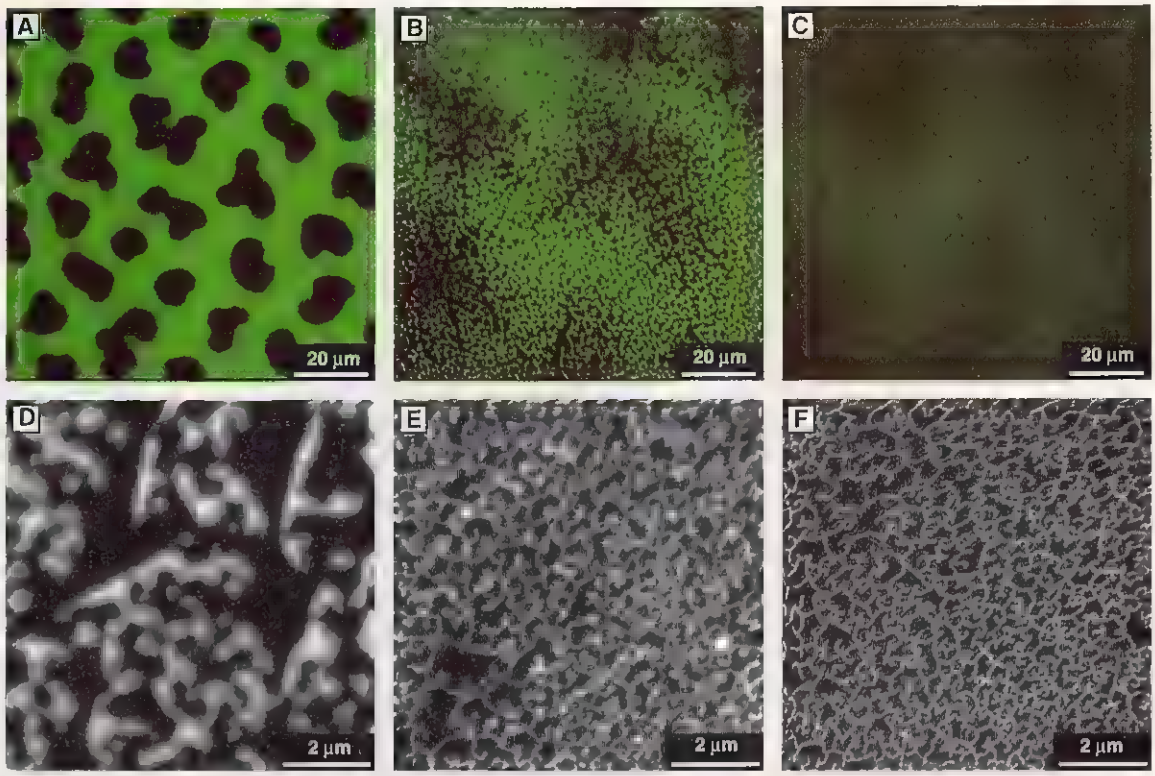

Fig. 2. (**A** through **C**) FFM images of DPPC/0.5 mol % Bodipy-PC monolayers sampled at three different pressures: (A) 7 mN/m, (B) 10 mN/m, and (C) 30 mN/m. (**D** through **F**) NSOM images of the same monolayers sampled at three different surface pressures: (D) 10 mN/m, (E) 20 mN/m, and (F) 30 mN/m. The web structure in (E) and (F) could reflect the presence of nanoscale crystals with hexagonally packed lipids, as observed by x-ray and electron diffraction from transferred and nontransferred lipid monolayers (*2*).

structure. This behavior has been the focus of several theoretical treatments (*4*).

Little is known about lipid monolayer structures at a scale between 10 nm and 1 μm. This has been largely the result of a lack of techniques operating in this range. Although AFM is able to disclose structures at this scale, it is limited to studying changes in the surface topology or, in rare cases, changes in the rheology of thin films. A more promising approach is to use near-field scanning optical microscopy (NSOM) (*8*, *9*). This technique combines the fluorescence contrast of conventional optics with spatial resolution as fine as 30 to 50 nm. Here we take advantage of these features to investigate the structure of lipid monolayers transferred from an air-water interface to a glass substrate under controlled surface pressure (*10*, *11*). The samples were first imaged by NSOM and then by FFM (*12*).

Pressure-area (πA) isotherms were obtained for samples of the following: (i) 99.5 mol % 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)/0.5 mol % Bodipy-PC, (ii) DPPC/0.5 mol % Bodipy-PC/1 mol %

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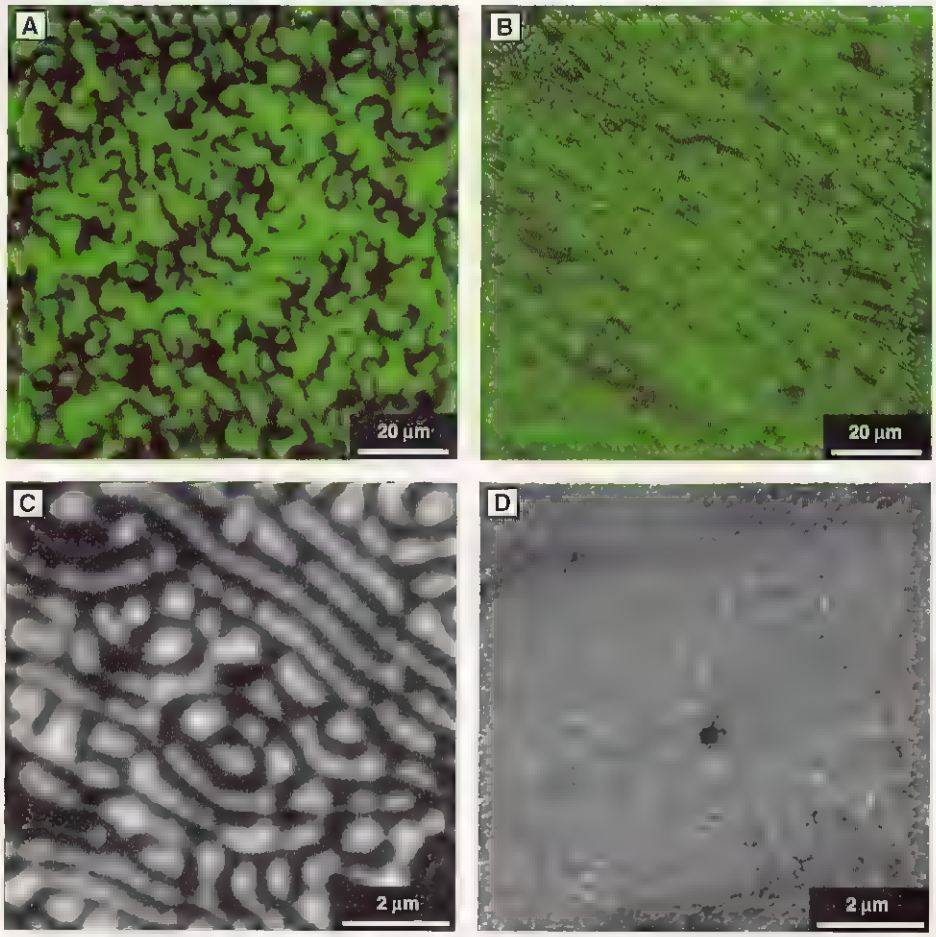

Fig. 3. FFM images of DPPC/Bodipy-PC/1.0 mol % cholesterol monolayers sampled at (**A**) 7 mN/m and (**B**) 10 mN/m. (**C**) NSOM images of the same monolayers transferred at 10 mN/m and (**D**) 20mN/m (*20*).

cholesterol, and (iii) DPPC/0.5 mol % Bodipy-PC/0.5 mol % ganglioside G_{M1} (Fig. 1). Four different regions corresponding to different lipid phases can be distinguished in the πA curves: (i) a liquid-expanded (LE) phase, (ii) a region of coexistence of LE and liquid-condensed (LC) phases, (iii) a LC-dominant (LCD) phase, and (iv) a solid-condensed (SC) phase.

Our FFM images of the DPPC/Bodipy-PC monolayer showed features of a single-component phospholipid monolayer phase transition (Fig. 2, A through C). Similar results have been reported for monolayers that were doped with other fluorescent probes that partition favorably into the LE phase within the LE/LC coexistence region (*5*, *6*, *11*). As the monolayer was compressed from the LE into the LE/LC coexistence region, small LC domains, which exclude Bodipy-PC, arose at various locations and then gradually grew in size. Some domains showed chiral features, as previously observed in similar systems (*13*). The sample transferred at pressure π = 10 mN/m exhibited a mottled structure with many fluorescent domains surrounded by a dark background (Fig. 2B). Frequently, these structures were too fine to be resolved by FFM. At even higher pressure, the samples were featureless as observed by FFM (Fig. 2C). In contrast, NSOM resolved many previously undescribed structural details, including uneven distribution of fluorescent molecules in the LE domains, gradual domain boundaries between the LE and LC regions (Fig. 2D), and a fine web structure with frequent intersections of ~120° (Fig. 2, E and F).

Low concentrations, 1 mol %, of cholesterol in DPPC monolayers changed the morphology of these lipid domains. In the region of LE/LC coexistence, cholesterol reduced the line tension energy between domains, so that long thin LC domains were formed (Fig. 3A). The shape and chirality of these domains were similar to those observed by FFM in nitrobenzoxadiazole-labeled phosphocholine (NBD-PC)/(*R*)-DPPC/cholesterol monolayers (*7*). As the surface pressure was increased to 10 mN/m, the domains further thinned and elongated (Fig. 3B). The NSOM images at the same pressure showed that the larger LE domains were connected by thin strings of LE lipid, which are too narrow and dim to be detected by FFM (Fig. 3C). Even though these strings have not been detected previously, their existence is consistent with current theories that suggest they arise in the presence of cholesterol from a reduction of the line tension energy, which opposes the dipolar energy of the LC domains (*4*, *7*). At higher pressure, NSOM images showed a compact web structure, which became more dense as the pressure was further increased (Fig. 3D).

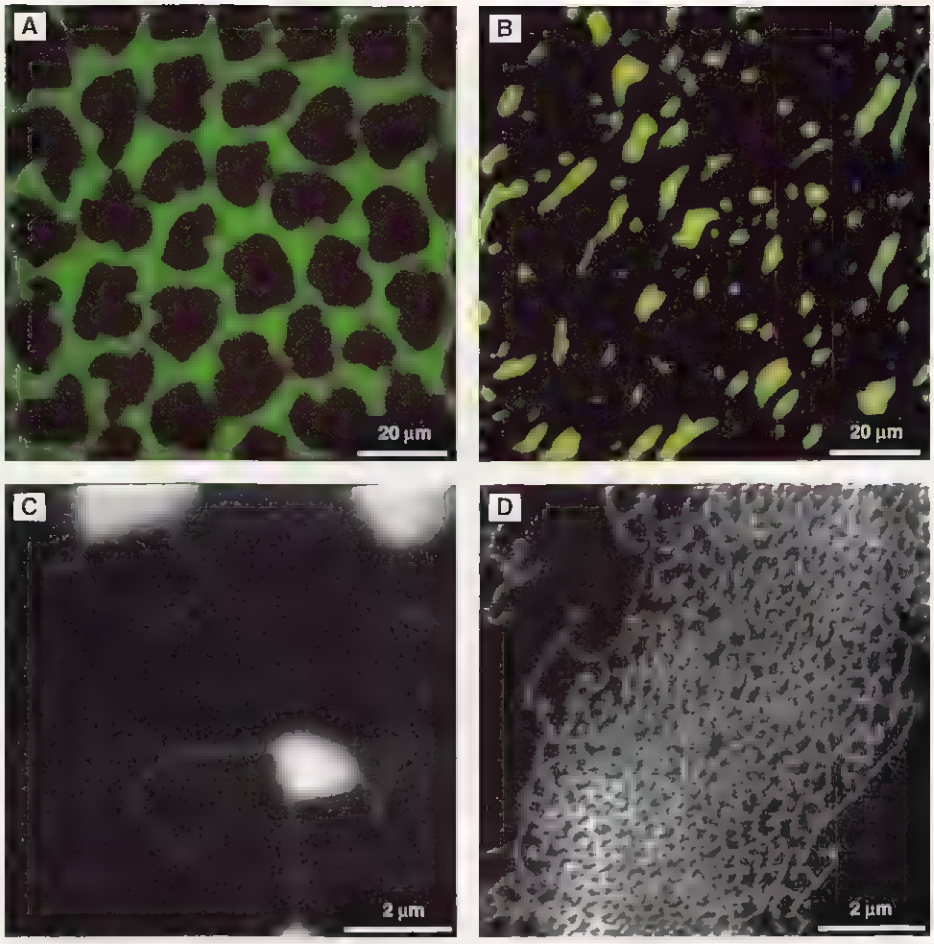

Fig. 4. FFM images of DPPC/Bodipy-PC/0.5 mol % ganglioside G_{M1} monolayers transferred at (**A**) 7 mN/m and (**B**) 10 mN/m. (**C**) NSOM images of the same monolayers transferred at 10 mN/m and (**D**) 20 mN/m.

Addition of 0.5 mol % of the ganglioside G_{M1} to the DPPC monolayer also had significant effects on the formation and structure of membrane domains. The dark LC domains that developed as the monolayer was compressed were faceted, grew to larger size than in the undoped DPPC/Bodipy-PC monolayers (compare Figs. 2A and 4A), and merged together, leaving residual LE phase domains between them (Fig. 4B). At 10 mN/m, the concentration of Bodipy-PC in these remaining LE domains was so high that the fluorescence spectrum shifted from green to yellow, as described elsewhere for Bodipy-PC in cell membranes (*14*). The corresponding NSOM image at 10 mN/m revealed fluorescent, thin "whiskers" stretching out from these bright domains (Fig. 4C). These whiskers may represent residual LE domains between LC domains that did not coalesce completely because of residual G_{M1} that remained trapped at the grain boundaries. At higher G_{M1} concentrations, 1 mol %, all domains were interconnected by whiskers. Thus, these interstitial regions likely represent domains of G_{M1} in the LE phase that are separated from domains of DPPC in the LC phase. As the pressure was increased to 20 mN/m, each fluorescent patch was again broken into a web structure whose density increased with pressure (Fig. 4D).

Besides resolving additional features in lipid monolayers, NSOM permits quantitative measurement of domain boundaries, monolayer composition, and the partition of the Bodipy-PC probe into the various phases. For example, NSOM showed that the fluorescence intensity gradually diminishes across the LE/LC domain boundary (Fig. 5), rather than exhibiting the sharp discontinuity of a simple phase boundary. This gradient can be explained in terms of a recent electrostatic model that demonstrates electric fields, near critical points, can induce concentration gradients in monolayers even without phase separation (*15*). According to this model, the concentration gradient depends on several parameters—such as the electric field gradient, molecular packing densities, and the dipole densities of lipid components—and is expected to change in response to variations in these experimentally controllable parameters. In fact, in

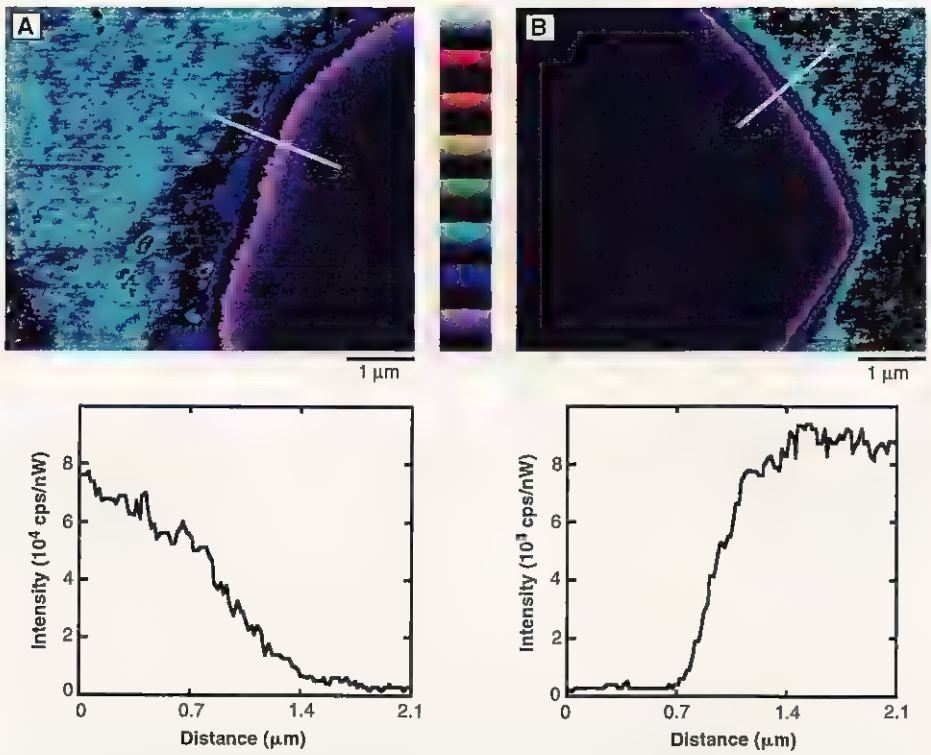

Fig. 5. Concentration gradient of Bodipy-PC molecules across the LE/LC domain boundary of $\pi = 7$ mN/m samples. (**A**) An NSOM image of the domain boundary region of a DPPC/Bodipy-PC sample and the intensity profile along the line. (**B**) An NSOM image and the intensity profile of a DPPC/0.5 mol % Bodipy-PC/0.5 mol % ganglioside G_{M1} sample. The signal intensity is normalized for the excitation power from the NSOM probe.

monolayers containing 0.5 mol % G_{M1} the gradient was steeper than in monolayers with only Bodipy-PC probe in DPPC (Fig. 5B). This gradient suggests that addition of G_{M1} molecules with negatively charged head groups alter these parameters either by screening dipole moments or by changing the molecular packing densities. We also found that the fluorescence-intensity gradient became steeper as the surface pressure of the monolayer was increased (*16*). This behavior is expected if the dipole moment of the Bodipy moiety is decreased when the long axis of Bodipy molecule is aligned perpendicular to the subphase surface as the packing density of the lipids increases.

Earlier work on the fluorescence imaging of single molecules with NSOM (*17*) provided a calibration of the instrument that allowed us to extract information on the monolayer composition and probe partitioning from histograms of the observed fluorescence intensities. Given the quantum yield of Bodipy-PC and an aperture radius of ~40 nm, we determined number densities in the LE phase of $\sim 5.8 \times 10^3$ and $\sim 1.3 \times 10^4$ Bodipy-PC molecules per square micrometer at pressures of 4 and 7 mN/m, respectively. These values are consistent with those calculated from the mole fraction of Bodipy-PC, the molecular area determined from the πA curve, and the fractional area of the LE phase as measured from FFM images (*18*).

The same analysis at higher pressures showed that during the transition from LCD to SC phase, Bodipy-PC probes are forced into the SC phase. The minimum intensity measured for the SC phase in samples at 20 mN/m corresponded to ~220 molecules/μm^2, shifting to ~760 molecules/μm^2 as the pressure increased to 30 mN/m. This analysis implies that at high pressures the SC phase is not completely free of fluorescent probe, even though Bodipy-PC partitions strongly into the less condensed phase. Intensity distributions measured for G_{M1}-containing monolayers demonstrated lower densities, ~60 and ~340 Bodipy-PC molecules/μm^2 at 20 and 30 mN/m, respectively, thereby indicating that G_{M1} suppresses the movement of Bodipy-PC molecules from the less condensed to the more condensed regions of the monolayer.

The high resolution and molecular sensitivity of NSOM might be used to study other issues involving lipid monolayers in addition to those discussed above. For example, the orientations of single molecules incorporated into domains of different phases could be measured to study tilt angles and molecular packing, and simultaneous fluorescent labeling for lipids and incorporated proteins could be used in the study of lipid-protein interactions and domain formation in model and cell membranes (*19*).

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10. We formed lipid monolayers at 21° ± 5°C by spreading a freshly prepared mixture of 99.5 mol % DPPC (Avanti Polar Lipids) and 0.5 mol % of the fluorescent lipid analog Bodipy-PC (Molecular Probes, D-3803) in chloroform-ethanol solution, at the air-water (Milli-Q, pH 5.5) interface of a Langmuir-Blodgett trough (NIMA, Coventry, UK) equipped with a Wilhelmy balance to measure the surface pressure of the monolayer. After allowing the solvent to evaporate for several minutes, the monolayer was compressed at a speed of 41 mm^2/s with pauses at different surface pressures to transfer samples of monolayer to glass substrates. Details of the method are described in L. K. Tamm, *Biochemistry* **27**, 1450 (1988). The FFM images showed that the acyl chain–labeled Bodipy-PC has the same partition behavior in the LE/LC coexistence region as does NBD-PC (Molecular Probes, N-3787).
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mixture of DPPC and Bodipy-PC molecules under the influence of the electric field, resulting in the concentration gradient of Bodipy-PC in LE domains near the boundary of LC domains. Even though the monolayers were sampled only from compression cycles and were not compared with the samples from expansion cycles, we believe that the partition of fluorescent molecules had reached equilibrium at the moment of transfer. The interval between compression and transfer (several minutes) was enough time for the distribution of fluorescent molecules to reach equilibrium through diffusion. The diffusion coefficient of DPPC in the LE domain is 10^{-7} to 10^{-8} cm²/s.

16. Averaged half-decay lengths (the lateral distances where the fluorescent intensities fall to half those of the maxima) determined from the cursor profiles across the domain boundaries for the monolayers sampled at π = 7, 10, 20, and 30 mN/m were 661 ± 133, 181 ± 38, 65 ± 15, and 37 ± 8 nm, respectively, in DPPC/Bodipy-PC samples and 345 ± 31, 143 ± 33, 57 ± 17, and 56 ± 13 nm, respectively, in the samples with an additional 0.5 mol % of ganglioside G_{M1}.
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21. We thank R. Chichester and R. Pagano for their valuable help and suggestions and H. McConnell for helpful discussions. Supported by NIH grants AI14584, DK44375 (M.E.), and AI30557 (L.K.T.).

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Rapid Clay Mineral Formation in Amazon Delta Sediments: Reverse Weathering and Oceanic Elemental Cycles

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Formation of aluminosilicate minerals in marine sediments was proposed over 30 years ago as a potentially important control on the chemistry of the oceans. Until now, this reverse weathering process has been largely discounted because of insufficient direct evidence for its existence. Experiments with unaltered, anoxic, Amazon delta sediments showed that substantial quantities of K-Fe-Mg clay minerals precipitated on naturally occurring solid substrates over times of ~12 to 36 months at ~28°C. A range of pore-water, solute-flux, and solid-phase criteria indicates that comparable clay mineral precipitation processes occur throughout Amazon shelf sediments, contributing ≥3 percent of the weight of the deposits and consuming ~10 percent of the global riverine K^+ flux.

The rapid formation of authigenic clay minerals during early sedimentary diagenesis was originally hypothesized as a likely process substantially influencing oceanic chemistry and closing a variety of elemental cycles through reverse weathering (*1*). The concept has not gained wide acceptance because of the lack of direct evidence for precipitation of such minerals in major deltas. Discovery of massive hydrothermal cycling of elements at midocean ridges has also decreased the obvious necessity for sedimentary sinks for certain solutes in geochemical budgets (*2*). However, problems concerning the geochemical balance of several major and minor elements still exist and can be overcome if early diagenetic formation of aluminosilicate minerals is assumed (*3*).

Authigenic glauconitic green clays form in small but concentrated amounts in continental shelf sands, upwelling areas, and sedimentary microenvironments, but such clays are usually considered relict, forming over thousands of years (*4*). Low-temperature authigenic smectites are also known to form from siliceous biogenic debris and metal oxides in local regions influenced by hydrothermal metal sources (*5*). Evidence for clay formation in nearshore depositional environments with high sediment accumulation rates has been indirect and has usually been inferred from observed trends in pore water solutes (K, F, Mg, and Al) or from small changes in solid-phase elemental compositions and operational leaches (*6–8*). In these latter cases, transported debris dominates accumulated material and makes documentation of disseminated authigenic clays difficult. In a few cases, direct evidence for nearshore early diagenetic clay formation (for example, the presence of nontronite, illite-smectite, and berthierine) has been found (*9*, *10*). The presence of authigenic clays documented to date in a range of environments therefore makes it certain that such minerals can form under the right conditions. The major questions that remain are whether the formation of such phases is rapid and whether it is geochemically significant.

As part of a general study of diagenetic processes in Amazon delta sediments, we investigated the potential formation of authigenic minerals during deposition (*11*). The Amazon River contributes ~6% of the total river particulates delivered annually to the oceans (*12*). Most of the Amazon river sediment is deposited on the adjacent continental shelf as a prograding delta. The suspended matter in the river is primarily of Andean origin (~82%) (*13*). The remainder is contributed by weathering in the Amazon drainage basin and consists of cation-poor (such as kaolinite and amorphous material) and cation-rich (such as smectite) aluminosilicate particles and of Si,Al,Fe oxides and oxyhydroxides as discrete particles and particle coatings (*10*, *14*). Upon entering the ocean, this material is mixed with reactive planktonic debris (organic carbon and SiO_2) and undergoes a variety of diagenetic changes, including extensive mobilization of Fe and Mn (*15*).

We simulated the conditions under which authigenic mineral precipitation must take place in a series of sediment incubation experiments that allowed ready separation of reaction products from the sedimentary matrix. To do this, we inserted small quantities (~0.5 g) of well-characterized solid substrates directly into otherwise unaltered Amazon delta sediments. Sediment was collected from the upper ~1 to 2 m of both inshore and offshore delta sites by means of box and kasten-type gravity corers. Except for possible diffusive exchange with overlying water or physical reworking by currents, material was subsequently maintained under conditions typical of burial in the delta. Substrates were (i) standard kaolinite, representative of the cation-poor aluminosilicate material that is one product of the tropical weathering regime of the Amazon basin; (ii) quartz sand grains, also a typical transported sediment component; (iii) FeOOH-coated quartz grains, representative of lateritic debris and commonly present in these sediments; and (iv) glass beads, simulating amorphous silica diatom frustrules, a biogenic product of photosynthesis in the water column that is deposited in Amazon delta sediments (*16*). Each substrate type was attached by a thin film of epoxy onto an acrylic slide, covered with a 0.4-μm nuclepore membrane filter and a nylon mesh outer screen, and inserted into the center of 250- to 1000-ml plastic bottles filled with natural Amazon delta sediment (wet and unaltered). Bottles were

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filled and sealed under N_2 and anoxically incubated in larger, sealed glass jars at 28°C for 12 to 36 months. The precipitation probe substrates were retrieved from the sediment under a N_2 atmosphere, washed with O_2-free filtered seawater, and briefly equilibrated with O_2-free distilled, deionized water in order to eliminate seawater salt precipitation during subsequent freeze-drying. Isolated substrates were stored under N_2 until further solid-phase analysis.

Under the scanning electron microscope (SEM), substrates (ii) and (iii) showed extensive precipitation of aluminosilicate material between substrate mineral grains as well as on both sides of the nuclepore filters. In the case of glass beads, precipitate formed predominantly on the filter membrane. Extensive dissolution of beads was found in all the experiments, and in many instances the glass had completely dissolved. The kaolinite grains were disintegrated but no enrichment in cations was evident from microprobe analysis. In the FeOOH-coated quartz substrates, we observed alteration of the external parts of the FeOOH coating to a Si-Al-Fe–rich phase with traces of other cations. This was probably the result of a reconstitution reaction similar to those documented in natural coated particles from the same area (*10*). Enrichment in P was also detected.

In the quartz grain and FeOOH-coated quartz grain substrates, the precipitate filled the space between the grains, forming a mesh constructed from 1- to 10-μm individual crystallites (Fig. 1A). The crystallites had a curved platy morphology (Fig. 1B) with extensive development of the *ab* plane. Transmission electron microscopy (TEM) observations showed that some of the platy crystals had a subeuhedral pseudohexagonal morphology (Fig. 2) (*17*). X-ray powder diffraction analysis (wavelength = 1.14857 Å, from a synchrotron source) of the precipitate indicated the presence of a 10.0 Å peak and a broad 7.16 Å peak. Higher order peaks are broad and are indicative of a disordered structure (*18*). Some alteration of the minerals due to dehydration during sample preparation (freeze-drying) is likely. Energy-dispersive system (EDS) analyses and single-crystal TEM-EDS spectra show Si, Al, Fe, K, and Mg as the predominant cations in most crystals. The Al, Fe, and K content varied substantially between individual crystals.

These observations indicate that the precipitates are clay minerals. We ascribe the 10.0 Å type to a dominant K-Fe–rich phase, which is consistent with a mica-type clay mineral. The 7.16 Å peaks probably belong to a less abundant Fe-rich, K-poor phase. We also conducted wave-dispersive system (WDS) electron microprobe analyses on precipitates. These analyses probably represent an average composition of a multicrystal assemblage rather than a single mineral crystal (*19*). Elemental analyses were converted to structural formulas with the assumption of a total anion charge of −44 typical of a mica-type clay mineral. The average structural formula is: $(K_{0.96}Na_{0.05})$-$(Al_{3.27}Fe^{+2}_{0.90}Mg_{0.39}Ti_{0.03})(Si_{6.47}Al_{1.53})$-$O_{20}(OH,F,Cl)_4$. We assume that most of the iron is present in the mineral structure in the form of Fe^{+2}, because the sediments are anoxic and are characterized by high concentrations of dissolved Fe^{+2}, a typical property of Amazon shelf sediments (*15*, *20*). Traces of Ca and Mn were also detected. NH_4^+, which is abundant in the pore waters, is probably also present in the clay but was not measured. Comparison with reported chemical compositions of other Fe-rich, K-rich, mica-type clay minerals such as glauconite (*21*) shows that the precipitates are depleted in Fe and enriched in Al.

The incubation experiments directly demonstrate the potential for rapid precipitation of clay minerals in Amazon delta sediments and indicate their likely structure and average composition (*22*). A variety of additional field evidence suggests that substantial disseminated authigenic clay that is consistent with the experimentally observed composition does indeed form. Pore water profiles in Amazon delta topset beds show depletion of F^- and K^+ with depth, despite rapid physical reworking of the upper ~1 to 2 m of the seafloor (*8*). On the basis of pore water transport models, direct measures of reaction rates, and operational solid-phase leaches, Rude and Aller estimated that ~7% of the riverine F^- supply to the oceans is taken up on the Amazon shelf and that formation or reconstitution of clay minerals is the most probable cause of both F^- and K^+ uptake (*8*). Estimated F/K flux ratios were in the general range expected for the occurrence of clay mineral precipitation.

The compositions of aluminosilicate material that we observed on the precipitation probes agree with these previous inferences. The F^- content of the experimental precipitates is in the range of 0.0146 to 0.3056 mole percent (mol%), with an average value of 0.148 mol%. The potassium content of the precipitates ranges from 3.9 to 11.0 mol%, with an average value of 6.98 mol%. Compared with other K-rich clays (transported illites), the neoformed clays are depleted in K. This apparent depletion could be the result of the presence of a K-poor phase that might have contaminated the analysis or could result from the presence of a mixed-layer clay mineral. Alternatively, it can be explained by a process similar to but more rapid than the one proposed by Odin and Matter for the neoformation of glauconite (*21*). They proposed that the first material precipitating from solution is poorly crystallized, K-poor, and Fe-rich with a high layer charge and that the precipitate evolves with time to a better crystallized K-enriched phase.

Simple mass balance calculations from the available fluorine flux data and the chemical composition of the precipitates indicate that neoformation of K-rich clay minerals in the Amazon delta has implications for the global K and F budgets. The

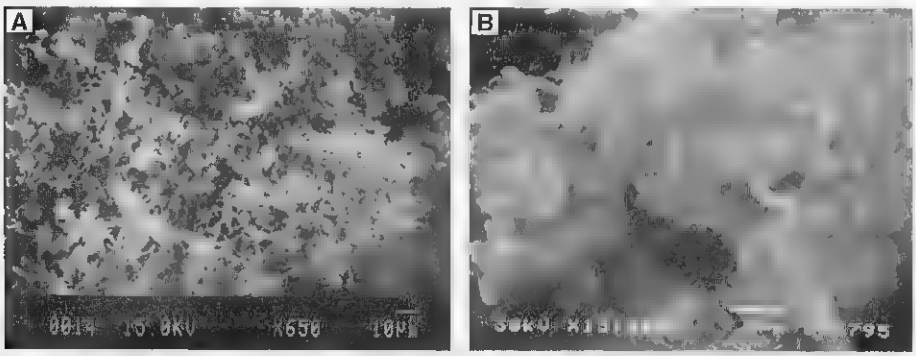

Fig. 1. (**A**) SEM picture of three-dimensional mesh precipitate of authigenic clay, formed between quartz sand substrates after ~18 months of incubation (scale bar, 10 μm). (**B**) SEM picture of authigenic clay crystallites demonstrating apparent monomorphological character with a curved flake shape (scale bar, 1 μm). The average structural formula given in the text was derived from microprobe analyses of comparable multiple crystals.

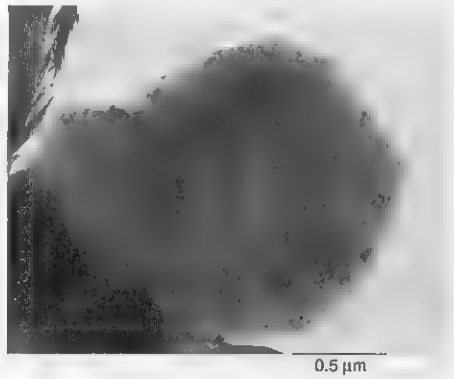

Fig. 2. Bright-field TEM image of Fe-rich precipitate. The crystallite exhibits a pseudohexagonal morphology and is composed of smaller pseudohexagonal crystals (scale bar, 0.5 μm).

flux of fluorine into the sediments predicted from pore water profiles (nonsteady-state minimum) ranges from 28 to 268 mmol m^{-2} day^{-1}. The estimated average annual fluorine sink in these sediments, calculated solely from aerially weighted pore water profile fluxes, is 2.6×10^9 mol $year^{-1}$ (23). The average F/K ratio measured on the neoformed clays is 21 mmol mol^{-1}, with a range of 4.4 to 41.37 mmol mol^{-1}. If this average compositional ratio holds for authigenic precipitates on the shelf, the annual K sink in Amazon continental shelf sediments amounts to 4.8×10^{12} g, representing ~10% of the annual riverine K supply to the oceans (24). This process of K uptake likely occurs in other depositional environments. Tropical river systems in general deliver ~60% of the continental particle flux to the oceans, and their muddy deltas may have similar diagenetic characteristics to that of the Amazon. Thus, the formation of K-rich clays in shelf environments with high sediment supply combined with processes of K uptake during low-temperature alteration of the upper oceanic crust (25) may contribute substantially to balancing the global budget of potassium.

Clay mineral formation and FeOOH coating alteration reactions require sources of both Al and Si. The incubation experiments demonstrate that Al must readily migrate in solution in order to pass the precipitation probe membranes. The source of Al must be the dissolution of relatively unstable amorphous Al oxides or other highly weathered aluminosilicate material (26). Because there is no evidence for unusually high concentrations of dissolved Al in Amazon delta sediments, there must be a close coupling of dissolution and reprecipitation reactions without buildup of dissolved Al intermediates. It is clear, however, that although Al may not migrate large distances, a portion is reactive. The common apparent immobility of Al does not preclude its involvement in near-simultaneous dissolution-precipitation processes.

There are a number of indications that the major limitation on authigenic clay mineral formation in Amazon delta sediments is likely to be the supply of reactive silica. The dissolution of glass beads in the precipitation experiments suggests that clay mineral precipitation results in substantial undersaturation of pore waters with respect to amorphous silica. Measured levels of dissolved silica in Amazon shelf pore waters are typically ≲200 μM over the upper ~1 m and often reach only ~300 μM at depths up to ~8 m, which are some of the lowest concentrations reported from marine sediments and are substantially below opaline silica solubility (Fig. 3A). In addition, diffusive fluxes of dissolved silica across the sediment-water interface are among the lowest ever measured in shallow marine environments and often show uptake from the water column, particularly in regions underlying turbid inshore waters of low productivity that are away from the dominant offshore sources of diatom debris (Fig. 3B). Although there is a large flux of diatomaceous debris to the bottom, little is preserved or buried as such (27). At least a portion of the solid biogenic silica flux may be converted into authigenic clay. The proposed control of biogenic silica over the amount of authigenic clays formed has implications for the geochemical budget of silica. If such control is confirmed in the future, it would result in the addition of authigenic clay formation to the known list of biogenic silica sinks in the oceans (28).

The pH of Amazon sediment pore waters is in the typical range of ~7.2 to 7.4 and is not particularly corrosive to siliceous debris. One possible mechanism for Si mobilization in the incubations and under field conditions comes from the known coupling of Fe redox cycling with Si (from quartz and diatom frustules) dissolution (29). This coupled mechanism is particularly viable in Amazon shelf sediments, where Fe cycling during organic matter remineralization dominates early diagenetic properties, and the extensive physical reworking of sediments causes repetitive redox oscillations, regenerating FeOOH. The dissolution-precipitation process, balanced for the stoichiometry of the neoformed clay minerals in Amazon delta sediments, is shown in Fig. 4.

As the Si, Al, and Fe reactant sources are essentially highly degraded weathering products, the general schematic reaction may be viewed as a form of reverse weathering. Reconstitution reactions of less degraded weathering products almost certainly also occur. These reactions likely result in dissemi-

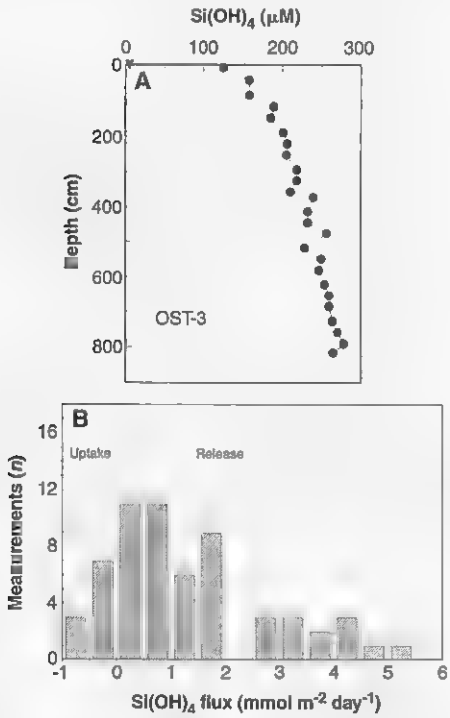

Fig. 3. (**A**) Representative pore water concentration profile of dissolved $Si(OH)_4$ in a piston core from station 4213, Open Shelf Transect 3 (OST-3), obtained from the high-accumulation-rate region of the Amazon delta topset deposits. Concentrations are typically 100 to 200 μM in the upper ~1 to 2 m of sediment over much of the delta topset region and tend to be ~300 μM at depth.The water column value (~5 μM) close to the sediment-water interface is shown by a solid triangle. (**B**) Frequency histogram of net diffusive fluxes of dissolved $Si(OH)_4$ across the sediment-water interface obtained by incubation cores (24 hours) at eight stations. Stations were sampled seasonally at four different times. Inshore stations often show net uptake of $Si(OH)_4$ from overlying water despite the absence of benthic photosynthesis. The mean annual flux from the delta deposits is ~1.3 mmol m^{-2} day^{-1}.

Release of reactants to solution (1)

$$6.47SiO_2 + 4.8Al(OH)_3 + 0.9Fe(OH)_3 + 0.225C_{org} + 3HCO_3^- + 12.49H_2O \rightarrow 6.47Si(OH)_4 + 4.8Al(OH)_4^- + 0.9Fe^{+2} + 3.225CO_2$$

Clay mineral neoformation (2)

$$6.47Si(OH)_4 + 4.8Al(OH)_4 + 0.9Fe^{+2} + 0.96K^+ + 0.39Mg^{+2} + 0.05Na^+ + 0.03Ti(OH)_4 + 1.19CO_2 \rightarrow (K_{0.96}Na_{0.05})(Al_{3.27}Fe^{+2}_{0.90}Mg_{0.39}Ti_{0.03})(Si_{6.47}Al_{1.53})O_{20}(OH)_4 + 1.19HCO_3^- + 20.01H_2O$$

Net reaction (3)

$$6.47SiO_2 + 4.8Al(OH)_3 + 0.9Fe(OH)_3 + 0.225C_{org} + 0.96K^+ + 0.39Mg^{+2} + 0.05Na^+ + 0.03Ti(OH)_4 + 1.81HCO_3^- \rightarrow (K_{0.96}Na_{0.05})(Al_{3.27}Fe^{+2}_{0.90}Mg_{0.39}Ti_{0.03})(Si_{6.47}Al_{1.53})O_{20}(OH)_4 + 2.035CO_2 + 7.52H_2O$$

Fig. 4. Likely schematic reactions that lead to the neoformation of clay minerals. SiO_2 derives from skeletal material or other sources (such as quartz dissolution during redox oscillation); $Al(OH)_3$ comes from Al-oxide dissolution. Aluminosilicate dissolution is another potential source of Al and Si. Even though dissolution of an aluminum phase was not directly observed in our experiments, previous studies from the same region have demonstrated that such a process occurs (6). Fe^{+2} is from Fe reduction coupled with organic matter oxidation; K, Na, and Mg come from seawater; and Ti comes from dissolution of a Ti-bearing solid phase. Comparable reaction schemes for the formation of authigenic nontronite and illite-smectite have been proposed for sediments in Kaneohe Bay, Hawaii (9). In this case, Mg, Ca, and (to a lesser extent) Na and K showed depletions in pore water profiles and were present in the inferred authigenic aluminosilicate phases. The aluminosilicate phases reacting were derived from basalt weathering on land.

nated authigenic clay minerals in a substantial proportion of highly weathered continental debris and have important implications for global controls of elemental cycling.

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16. Substrate (i) was Ward's kaolinite standard no. 9, collected from Mesa Alta, NM. Substrate (ii) was quartz sand, grain size 200 to 400 μm. Substrate (iii) was quartz sand grains coated with a FeOOH-gel made from $FeCl_3$ and NaOH at a pH of 5. The resulting coating was x-ray amorphous, and the probable compound was FeOOH. For substrate (iv), the beads were made out of glass composed mostly of Si and containing some Ca, Na, and Mg. Size range was 20 to 60 μm.
17. Submicron crystallites composed of Si, Al, K, Fe, and Mg and having a curved morphology were ubiquitous under the TEM.
18. We did not analyze lattice spacings (d) larger than 10.2 Å. Because of the small amount of material available for x-ray diffraction analysis, we used a capillary tube sample holder and a synchrotron radiation source at Brookhaven National Laboratory. The wavelength used in conjuction with the geometry of the sample holder–detector setup did not allow data acquisition at low angles. Powder diffraction patterns also showed the presence of amorphous material.
19. It is possible that the presence of any K-poor phase might have contaminated the analyses of the K-Fe–rich phase. We also cannot exclude the possibility of mixed layering. In addition, it is difficult to assess the contribution from the amorphous material present in the precipitates. More than 90% of the analyses were dominated by the inferred K-Fe phase.
20. The pH of the sediments after the termination of the experiments was within the range of the in situ values measured during collection (7.2 to 7.4). Analysis of the pore waters after the termination of the experiments showed that Mg, Ca, Si, Fe, and total CO_2 concentrations were in the range measured in shelf pore waters.
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30. We thank R. Reeder for obtaining the TEM data, M. Kunz and J. Parise for obtaining the synchrotron powder diffraction data, and G. Symmes for assisting with the microprobe analyses. P. Rude, M. Green, and J. Mackin helped with the probe construction and experimental setup. P. Bartholomew assisted with SEM-EDS analyses. The participants of AMASEDS assisted in all aspects of the field work. Financial support was provided by NSF (R.C.A). A fellowship from the State Scholarship Foundation of Greece to P.M. is acknowledged.

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Limits to Relief

Kevin M. Schmidt* and David R. Montgomery

Comparison of slope profiles in areas exhibiting widespread bedrock landsliding with the use of a model for the maximum size of stable hillslopes established that mountain-scale material strength can limit topographic relief. Conventional laboratory values for intact rock greatly exceeded integrative rock strength properties that were back-calculated from the upper limit to hillslope relief and gradient in the northern Cascade Range and Santa Cruz Mountains. Back-calculated strength values, however, were indistinguishable from those obtained through field and conventional laboratory measurements on the weakest members of each rock formation, as well as on glacial sediments along the Cascade front. These results contrast with the conventional assumption that relief is incision-limited and indicate that the relief of mountain ranges can reflect landscape-scale material strength, as well as the interaction of tectonic and climatic processes.

Relief is a fundamental landscape attribute that is widely recognized as reflecting the interplay of uplift and erosion (*1*). The role of material properties in relief development, however, is poorly understood. The conventional view that the relief of natural landscapes is incision-limited (*2*) reflects the observation that hillslope stability analyses using intact rock strengths predict the stability of cliffs kilometers in height (*3*). However, rock mass strength decreases with increasing spatial scale, because of the influence of spatially distributed discontinuities (*4*), and it has been unclear whether mountain-scale rock strength might be low enough to limit relief in bedrock landscapes (*1*). Through a regional field test of a slope stability model, we demonstrate here that mountain-scale material strength can limit relief development in bedrock landscapes.

A model for bedrock landsliding provides a framework for prediction of the maximum size of stable hillslopes or mountain fronts and thereby for the evaluation of the influence of material properties on relief development (*5*). As hillslope relief (H) increases, topographically induced gravitational shear stress across potential failure surfaces increases until it exceeds material strength and landsliding ensues. Culmann's two-dimensional, limit-equilibrium, slope stability model (*6*), which has been widely applied to unconsolidated deposits (*7*), predicts a bounding relation between hillslope gradient (β) and relief such that the maximum hillslope height (H_c) is given by

$$H_c = \frac{4c}{\gamma} \frac{\sin\beta \cos\phi}{[1 - \cos(\beta - \phi)]} \quad (1)$$

where c is cohesion, γ is unit weight, and ϕ is the internal friction angle. Hoek and Bray (*8*) modified Eq. 1 to incorporate pore water pressure, and Schmidt (*9*) integrated seismic accelerations. Assigning a representative γ, we used Eq. 1 to calculate landscape-scale c and ϕ from the upper limit to the range of β and H within a landscape. The actual material properties of incision-limited landscapes will exceed back-calculated values of c and ϕ, which indicates that bedrock strength could support deeper val-

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leys. In strength-limited landscapes, however, back-calculated and actual material properties should be equivalent, such that observed combinations of β and H define a limit to topographic development (LTD) beyond which incision of valley bottoms will induce bedrock landsliding that lowers peak elevations.

We analyzed hillslopes composed of the Eocene Chuckanut Formation and the overlying Quaternary glacial sediments located in the western Cascade Range of Washington state, as well as a sedimentary sequence in the Santa Cruz Mountains of central California. Widespread deep-seated landsliding in each study area implies that the observed relief approaches the LTD. We used aerial photograph analyses and earlier mapping (*10*) to identify deep-seated landslides; field surveys and measurements from topographic maps defined β and H. For each geologic sequence, we made a suite of transects from ridgetop to valley bottom to establish the local relief and maximum gradient (*11*). We fit the LTD for each unit to the upper envelope of β and H and used these back-calculated strength properties to predict the LTD both under saturated conditions and for horizontal seismic accelerations of 0.6*g*.

In Washington state, we mapped from aerial photographs over 585 km^2 along the United States–Canada border, an area encompassing the low-relief San Juan Islands as well as the higher relief landscape near the western flank of Mount Baker. We identified 34 mountain-front–scale bedrock landslides within the Chuckanut Formation (*9*), a sequence of alternating intervals of coarse- and fine-grained alluvial strata (*12*). Tertiary deformation compressed the sequence into broad northwest-plunging folds and high-angle faults. We differentiated hillslopes into dip and anti-dip slopes because large-scale structural anisotropy provides a strong control on hillslope strength (*13*). A plot of β versus H for measured transects revealed an arcuate upper bound characterizing the maximum observed topographic expression (Fig. 1). The LTD defined by the upper limit of the observed data for anti-dip slopes yielded $\phi = 21°$ and $c = 150$ kPa (Fig. 1A), whereas the threshold for dip slopes defined lower strength values of $\phi = 17°$ and $c = 120$ kPa (Fig. 1B). In situ measurements with a variably loaded sheargraph (*14*) on weak siltstone interbeds produced comparable strength values of $\phi = 27°$ and $c = 26$ kPa (*9*).

Quaternary glacial sediments in northwestern Washington on the west flank of the Cascade Range comprise a varied sequence of stiff, laminated lacustrine clay overlain by outwash sand and gravel and subsequently capped by boulder till. We treated the sequence as a single material unit in order to characterize the regional topographic development within glacio-fluvial deposits occupying valley floors. Within this unit, landslide thicknesses typically constitute a substantial proportion of relief, and the movement of landslide toes away from the hillslope face decreases post-failure hillslope gradients. Where possible, we reconstructed pre-landslide geometries from adjacent stable hillslopes. The LTD defined by β and H values from 178 field-surveyed transects along four channels (Middle Fork of the Nooksack River, Clearwater Creek, Rocky Creek, and Boulder River) yielded back-calculated strength values of $\phi = 29°$ and $c = 20$ kPa (Fig. 1C). Mean in situ properties measured with a variably loaded sheargraph in sandy silt outwash deposits were $\phi = 36° \pm 11°$ and $c = 12 \pm 9$ kPa (*9*).

The Santa Cruz Mountains of central California experienced widespread landsliding during the (magnitude) 7.1 Loma Prieta earthquake in 1989 (*15*). Landslides were concentrated in the high-relief, moderate-gradient, mountainous epicentral region as well as along the relatively low relief, high-gradient coastal cliffs along Monterey Bay. We measured hillslope attributes from topographic maps for 82 coseismic landslides in a sequence of lithologically similar marine sedimentary rocks from the Eocene to the Pliocene (*16*). The resulting LTD for the dry state yielded strength values of $\phi = 20°$ and $c = 60$ kPa. For comparison, triaxial and direct shear tests on the highly fractured, variably weathered, interbedded, and sheared sandstone, siltstone, and shale identified in slip surfaces of deep-seated landslides provided estimates of actual material strength controlling landslide displacement. Averaging values reported for fractured shale (*17*), the most common slide-plane material identified in drill cores, produced mean values of $\phi = 20° \pm 6°$ and

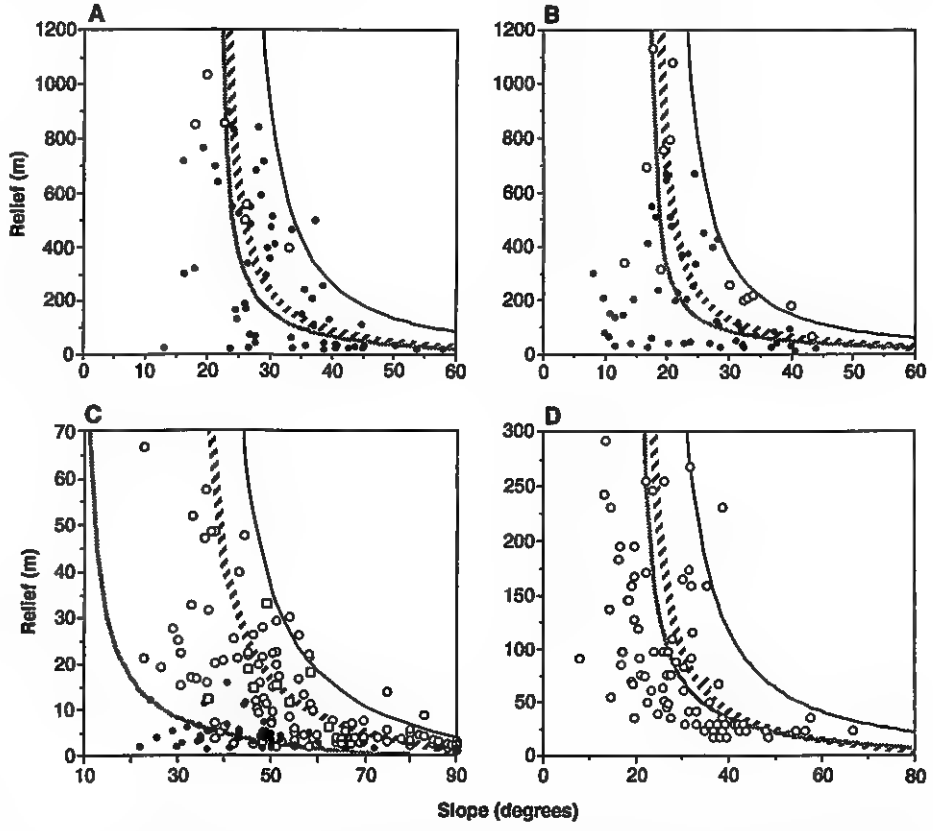

Fig. 1. Gradient versus relief for stable hillslopes (●), for landslide sites (○), and for reconstructed prefailure geometry of landslide sites (□) composed of three geologic units: the Eocene Chuckanut Formation, Quaternary glacio-fluvial sediments, and Eocene-to-Pliocene sediments of the Santa Cruz Mountains. Three LTD thresholds are depicted for each unit: dry conditions (solid black curve), saturated conditions (thick hatched curve), and horizontal seismic accelerations of 0.6*g* (gray curve). The LTD for the dry case was fit to the upper envelope of observations. Back-calculated strength properties from the dry case were used to investigate the influence of transient fluctuations caused by extreme pore pressures and seismic accelerations (*8, 9*) that temporarily suppress the LTD. **(A)** Anti-dip slope transects for the Chuckanut Formation measured from 1:24,000-scale topographic maps. Back-calculated strength properties are $\phi = 21°$ and $c = 150$ kPa. **(B)** Dip slope transects for the Chuckanut Formation measured from 1:24,000-scale topographic maps. Back-calculated strength properties are $\phi = 17°$ and $c = 120$ kPa. **(C)** Transects for Quaternary glacio-fluvial deposits surveyed with a hand level, a stadia rod, and tape. Back-calculated strength properties are $\phi = 29°$ and $c = 20$ kPa. Extreme suppression of seismic LTD results from the assumption of coseismic failure plane generation. **(D)** Landslide sites for marine sedimentary units of the Santa Cruz Mountains. Back-calculated strength properties are $\phi = 20°$ and $c = 60$ kPa.

$c = 69 \pm 32$ kPa. Similarly, mean strength properties for all lithologies (shale, siltstone, sandstone, and clay) were $\phi = 30° \pm 14°$ and $c = 69 \pm 32$ kPa (*17*).

Large earthquakes commonly trigger landslides over areas of 10^5 to 10^6 km^2 (*18*). To predict the degree of earthquake-induced LTD suppression, we modeled seismic accelerations as horizontal body forces oriented out of the hillslope face (*9*). The relative timing of failure plane generation, either aseismic or coseismic, is crucial to the degree of LTD suppression. We suspect that most landslides within unconsolidated glacial sediments form coseismically, with slip surface inclinations dictated by the strong motion magnitude. In contrast, failure planes within the Chuckanut Formation and formations of the Santa Cruz Mountains are considered to form progressively. Suppression of the LTD under horizontal accelerations of 0.6*g* exceeds that for complete saturation (Fig. 1). Glacial sediments exhibit the highest degree of LTD suppression (Fig. 1C) because failure surfaces are considered to form coseismically (*9*). Hence, the model implies that incised glacial deposits are prone to extensive landsliding during earthquakes. The combination of earthquake-induced landslides with measured seismic accelerations in the epicentral region of the 1989 Loma Prieta earthquake provides an opportunity to test model predictions. Four strong-motion instruments located throughout the epicentral region recorded free-field peak horizontal accelerations from 0.47 to 0.64*g* during the main shock of 17 October 1989 (*19*). Although LTD suppression under horizontal accelerations of 0.6*g* roughly agrees with the landslide distribution (Fig. 1D), the presence of landslides below the seismically suppressed regional LTD may reflect the long-term impact of repeated seismic disturbance, which loosens rock masses and reduces hillslope shear resistance, in addition to spatially variable rock properties and saturation frequency.

The close agreement between strength parameters back-calculated from the LTD method and those derived from field and laboratory tests of the weakest members in a rock mass indicates that large-scale rock strength may control the regional LTD. Values for material properties back-calculated from the LTD approach are lower than those determined by traditional laboratory analyses on intact samples of coherent material but agree with values reported for unconsolidated materials and for the weakest members of a rock mass (Table 1). Although high c values are common for intact rock, material discontinuities can dramatically reduce bulk cohesion. Properties determined from the LTD for the Santa Cruz Mountains are equivalent to triaxial and direct shear tests (*17*) on fractured shale beds within landslide slip surfaces (Table 1). Additionally, in situ sheargraph tests on glacial sediments yield values comparable to those given by the LTD approach. This agreement between disparate methods supports the fundamental approach of the LTD model.

Although representation of mountain-scale material properties is essential to the prediction of landscape development and landslide hazards, a stark dichotomy exists between the spatial and temporal scales of conventional laboratory analyses and their application to geologic problems. Small samples of intact rock used in laboratory tests yield strength parameters that are stronger than those of the entire rock mass, because the shear surface is restricted to a narrow sampling of the available discontinuities. Furthermore, apparent rock strength depends on applied stress rate (*20*). Typical strength tests thus overestimate material strength, because the duration of applied stress is orders of magnitude shorter and the failure plane is orders of magnitude smaller in surface area than are those for natural hillslopes.

No theory exists for independently predicting the specific LTD for a mountain range, but regions with considerable relief and steep gradients, such as active tectonic areas with rapid rock uplift, typically display widespread bedrock landsliding (*21*). In these areas, rock mass strength rather than valley incision rates may limit relief development. This control on landscape evolution is crucial to understanding the relation between climate, valley incision, and the uplift of mountain peaks. The attribution of late Cenozoic uplift of mountain ranges to increased valley incision and subsequent isostatic compensation (*22*), for example, assumes that relief can be increased by the incision of deep valleys. The similarity between back-calculated, large-scale, rock mass strength properties and engineering tests on the weakest members of rock formations implies that bedrock landsliding could inhibit valley incision, especially in mountain ranges composed of highly deformed or weak rock. Although it is recognized that relief reflects the interaction of valley incision and rock uplift, our results indicate that landscape-scale rock strength may also limit the relief of mountain ranges.

Table 1. Strength properties back-calculated from topographic expression (LTD), measured in situ with a sheargraph (*9*, *14*) and derived from laboratory tests (*8*, *17*).

Material	Friction angle (ϕ) (degrees)	Cohesion (c) (kPa)	Source
Chuckanut Formation (anti-dip slope)	21	150	LTD (this study)
Chuckanut Formation (dip slope)	17	120	LTD (this study)
Chuckanut Formation (siltstone)	27	26	In situ sheargraph measurement (*9*)
Hard sedimentary rock (sandstone)	35 to 45	10,000 to 30,000	Laboratory experiments (*8*)
Soft sedimentary rock (shale or coal)	25 to 35	1,000 to 20,000	Laboratory experiments (*8*)
Santa Cruz Mountains	20	60	LTD (this study)
Santa Cruz Mountains (shale)	20 ± 6	69 ± 32	Laboratory experiments (*17*)
Santa Cruz Mountains (shale, siltstone, sandstone, and clay)	30 ± 14	69 ± 32	Laboratory experiments (*17*)
Glacial sediments	29	20	LTD (this study)
Glacial sediments (sandy outwash)	36 ± 11	12 ± 9	In situ sheargraph measurement (*9*)

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Megascopic Multicellular Organisms from the 1700-Million-Year-Old Tuanshanzi Formation in the Jixian Area, North China

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Hundreds of specimens of megascopic carbonaceous fossils shaped like leaves have been found at the ~1700-million-year-old Tuanshanzi Formation of the uppermost Paleoproterozoic Changcheng Group (1600 to 1850 million years old) in the Jixian area, north China. These leaflike fossils mostly resemble the *Longfengshania*; each consists of a blade (with spoonlike, lanceolate, or ribbonlike shapes) with a single stipe, a holdfast, or both. On the basis of their megascopic dimensions, preliminary differentiation of organs or tissues, and possible remains of multicellular structures, they are benthic, multicellular algal fossils similar to the longfengshanids. These fossils indicate that multicellular organisms originated at least 1700 million years ago.

The emergence of multicellular organisms (metaphytes and metazoans) is an important event in the evolutionary history of Precambrian life since the emergence of unicellular eukaryotes. The oldest remains of multicellular organisms are therefore one of the keys to the early evolution of life on Earth. A few megascopic carbonaceous films of *Tyrasotaenia* from the 1700-million-year-old Tuanshanzi Formation of the Changcheng Group in the Jixian area, north China, have been reported (*1*) but have not been widely accepted as multicellular organisms (*2*, *3*).

In addition to a few samples of ribbonlike and sausagelike megafossils resembling the vendotaenids and tawuids, respectively, we have recently found more than 300 specimens of megascopic carbonaceous fossils shaped like leaves from the locality and horizon close to that described in (*1*). These leaflike megafossils have obvious characteristics of multicellular algae. The megascopic carbonaceous remains were found near Tuanshanzi Village and its adjacent area (40°10′N, 117°27′E), about 20 km northeast of Jixian Town (Fig. 1). The best-preserved specimens came from the lower part (first member) of the Tuanshanzi Formation, ~44 to 47 m above its base (Fig. 2). The Tuanshanzi Formation belongs to the Paleoproterozoic Changcheng Group. The group includes the Changzhougou (conglomerate, sandstone), Chuanlinggou (silty and illitic shale), Tuanshanzi (muddy and silty dolomicrite), and Dahongyu (sandstone, volcanic rocks, cherty dolomicrite) formations in ascending order, and has a total thickness of

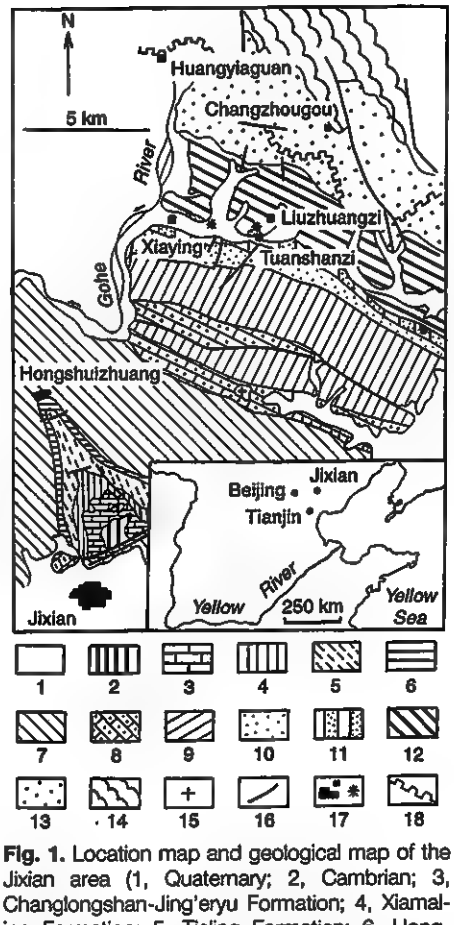

Fig. 1. Location map and geological map of the Jixian area (1, Quaternary; 2, Cambrian; 3, Changlongshan-Jing'eryu Formation; 4, Xiamaling Formation; 5, Tieling Formation; 6, Hongshuizhuang Formation; 7, Wumishan Formation; 8, Yangzhuang Formation; 9, Gaoyuzhuang Formation; 10, Dahongyu Formation; 11, Tuanshanzi Formation; 12, Chuanlinggou Formation; 13, Changzhougou Formation; 14, Archean; 15, Mesozoic granite; 16, fault; 17, town, village, and new fossil occurrences; and 18, Great Wall).

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2629 m. These rocks were deposited from ~1850 to 1600 million years ago (Ma) (*4*). The type section of this group is at Tuanshanzi Village and surroundings.

The Tuanshanzi Formation is mainly composed of muddy and silty dolomicrite with a total thickness of 518 m (*5*). The lower member of this formation is rich in muddy, silty, and carbonaceous lithologies, and has pyrite impregnations and black carbonaceous films on the bedding planes. In the upper member, the content of clastics increases markedly upward and dolomitic sandstone, sandy dolostone, thin-bedded sandstone, and small stromatolite bioherms are common. The lower member of the Tuanshanzi Formation is characterized by even bedding with local slumps. In the upper member, flute casts, furrow casts, ripple marks, mud cracks, and salt pseudomorphs are common. The Tuanshanzi Formation represents an upward shallowing sequence; its lower part was mainly formed in a relatively quiet, weakly reducing, subtidal environment (lagoonal facies), whereas its upper part formed in an unstable environment from the intertidal zone to the supratidal zone.

A whole-rock U-Pb isochron age of ~1776 Ma was obtained on a dolostone from the upper part of this formation in the Jixian area (*6*). More recently, a U-Pb isochron age of 1683 ± 67 Ma was obtained on a single zircon from volcanic rocks (lava) of the middle part of this formation in an area closely adjacent to Jixian (*7*). In addition, a U-Pb isochron age of 1625.3 ± 6.2 Ma was obtained on a single zircon from volcanic rocks (lava) of the overlying Dahongyu Formation in the Jixian area (*8*). The age of the lower part of the Tuanshanzi Formation, therefore, is ~1700 Ma.

The leaflike megafossils from the Tuanshanzi Formation all were found in dark gray to black muddy dolostones of the lower part of the formation and are distributed as black carbonaceous films (or, in some cases, brown casting molds), dispersed or in clusters on the bedding planes. They are abundant and most of them are well preserved. The carbonaceous films constituting megafossils are shaped like unbranched leaves or typical thallophytes; each typically consists of a sheetlike blade that has a single ribbonlike stipe, a rhizoidal holdfast consisting of many hairlike haptera, or both. The blades of the leaves have different shapes and sizes, although each kind of blade is roughly uniform. The spoonlike (type 1), lanceolate (type 2), and ribbonlike (type 3) shapes are most common (Fig. 3). The carbonaceous blades have different dimensions; most are 0.5 to 3.5 mm in width and 5 to 10 mm in length, whereas a few of the larger blades are >10 mm wide and some tens of millimeters long (Table 1). In addition, some smaller blades have also been seen in the fossiliferous horizons; the width of these smaller blades is 0.05 to 0.3 mm.

Although the interior and fine detailed structure have not been preserved in most of the carbonaceous leaves, some specimens show fine longitudinal striations on their surfaces as well as palisadelike structures. The palisadelike structures occur at the margins of specimens and may be paraphyses or sporangia (Fig. 4, A through C). In addition, some remains of interior multicellular structure, such as a mucilage canal and the multicellular structure surrounding it (Fig. 4, D and E) and other degraded multicellular structure (Fig. 4F), can be found in a few portions of specimens by scanning electron microscopy.

On the basis of these characteristics, we can draw the following conclusions about the biological affinity of the Tuanshanzi leaflike carbonaceous films. Although the carbonaceous films have different shapes, each of them can be found repeatedly, and collectively they show obvious stability in morphology. In addition, the spectral analysis shows that some large organic molecules (aggregated by aromatic hydrocarbon, ester, CH_2, or CH_3 groups) are present. Therefore, the films constitute fossils of old organisms and are not general carbonaceous fragments. Because these fossils are shaped like leaves and exhibit structural features of leaves, they are compressions of primitive plants and not primitive animals. The fossils not only have megascopic size and show preliminary differentiation of organs (into a blade and a single stipe or a holdfast), but also show some evidence of multicellular structures, including possible

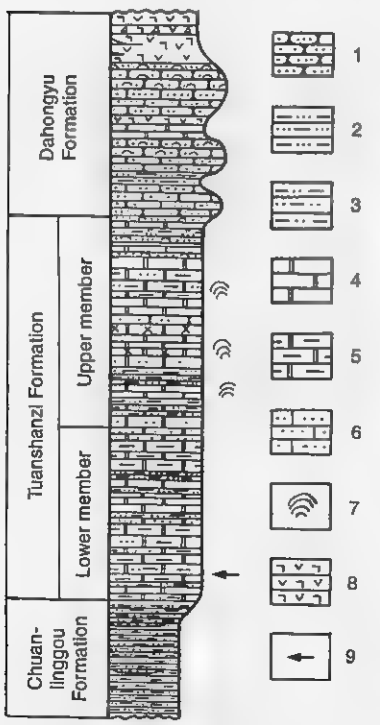

Fig. 2. Generalized lithostratigraphic column of the Tuanshanzi Formation in the Jixian area (1, sandstone; 2, argillaceous siltstone; 3, silty shale; 4, dolostone; 5, muddy dolostone; 6, silty dolostone; 7, stromatolites; 8, volcanic rocks; 9, fossiliferous horizon)

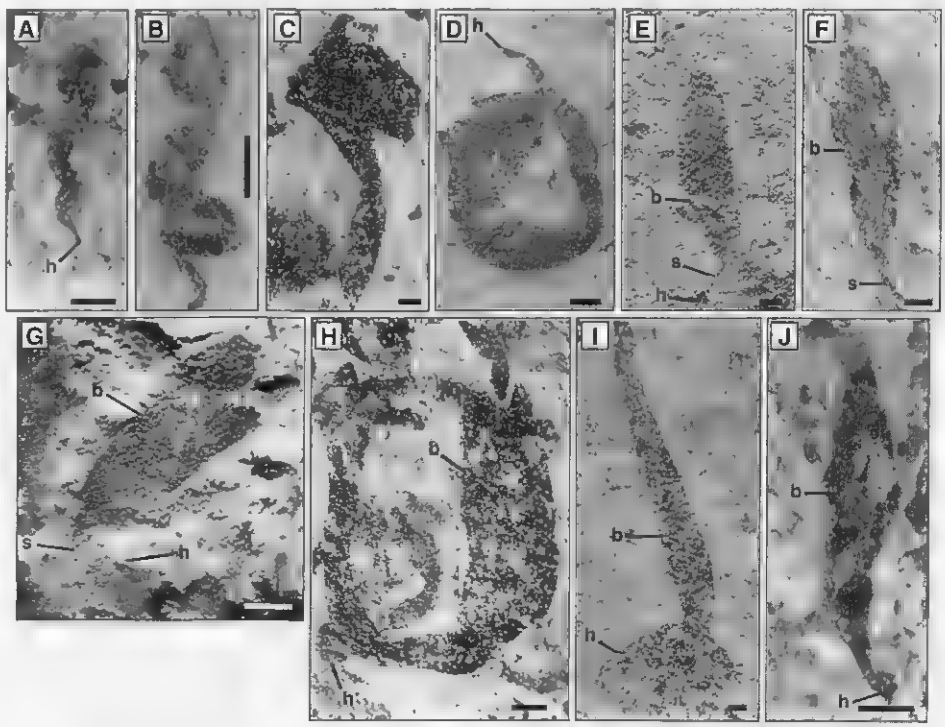

Fig. 3. Leaflike megafossils from the Tuanshanzi Formation of the Paleoproterozoic Changcheng Group (b, blade; s, stipe; and h, holdfast). (**A** through **D**) Spoonlike type (type 1); (**E** through **G**) lanceolate type (type 2); (**H** through **J**) ribbonlike type (type 3). Scale bars, 1 mm.

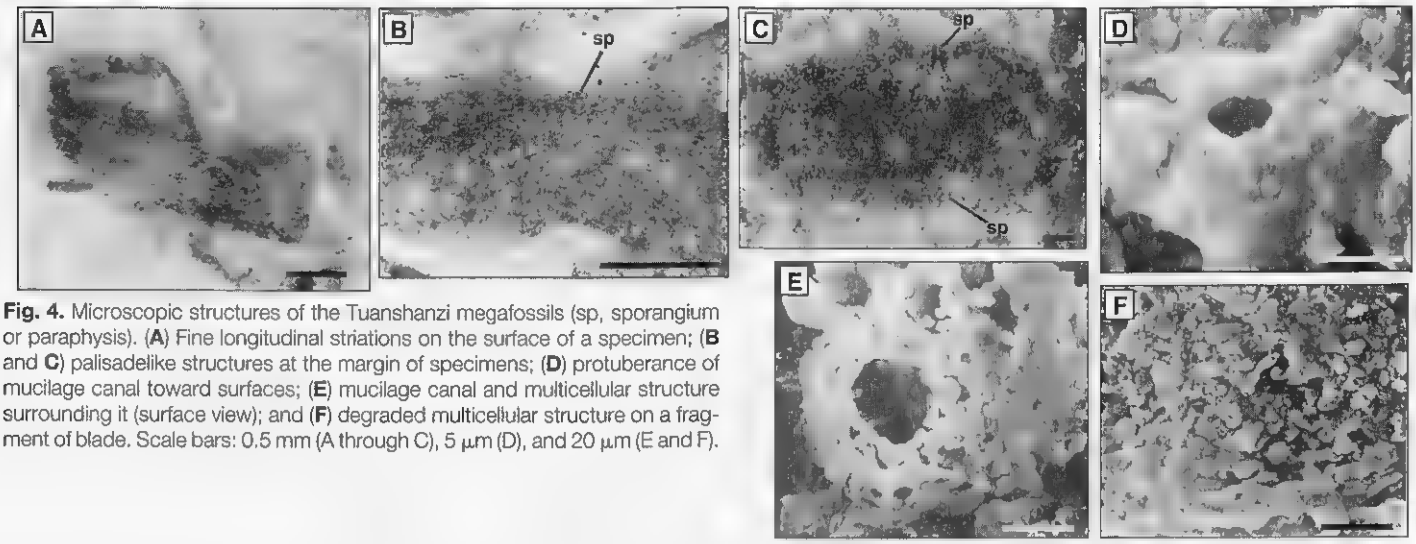

Fig. 4. Microscopic structures of the Tuanshanzi megafossils (sp, sporangium or paraphysis). (**A**) Fine longitudinal striations on the surface of a specimen; (**B** and **C**) palisadelike structures at the margin of specimens; (**D**) protuberance of mucilage canal toward surfaces; (**E**) mucilage canal and multicellular structure surrounding it (surface view); and (**F**) degraded multicellular structure on a fragment of blade. Scale bars: 0.5 mm (A through C), 5 μm (D), and 20 μm (E and F).

sporangia. Thus, these fossils can be excluded from the prokaryotes and their aggregates (*2*) as well as from the protists generally, and they should possess the affinity of benthic, multicellular organisms, that is, metaphytes.

Except for the spoonlike type, the blades of most of the fossil leaves vary between lanceolate and ribbonlike in morphology and resemble a large category of longfengshanid remains represented by *Longfengshania* (*2*, *9*). With respect to modern multicellular lower plants, the fossils are most similar to (but smaller than) the Laminariales, especially the Laminariaceae of brown algae (Phaeophycophyte), whose sporophytes not only have typical multicellular structures (with mucilage canals, hyphae filamentous structure, and a palisadelike structure consisting of unilocular sporangium and paraphyses) but are usually differentiated into a cylindrical stalk or stipe and one or many lanceolate to ribbonlike blades or laminae (*10*). Thus, the most leaflike fossils from the Tuanshanzi Formation are possibly fossils of brown algae closely related to the order Laminariales. The relation of the spoonlike fossils to modern algae is not yet clear. Although they resemble certain green algae such as *Ulvaria* in outline, they more likely are related to the brown algae (Scytosiphonales) on the basis of their palisadelike structure composed of sporangium and multicellular (?) filaments at the margins of the specimen (Fig. 4C).

These fossils are preliminarily defined as benthic, eukaryotic, multicellular metaphytes, possibly dominated by ancient brown algae. The oldest reported megascopic carbonaceous fossil, *Grypania spiralis* (Walter), was a corkscrew-shaped, spaghettilike organism from the 2100-million-year-old Negaunee Iron Formation, Michigan (*11*); however, although a eukaryote interpretation of *Grypania* seems most likely, a prokaryotic origin cannot be excluded (*12*). Most Precambrian carbonaceous megafossils are not confidently interpreted as multicellular algae, except for the longfengshanid films (*2*, *9*) and a number of branched fossils recently reported from the Upper Sinian (Vendian) of China (*13*). Because the longfengshanids and some other carbonaceous multicellular organisms (for example, *Tawuia*) are known from the Neoproterozoic, it has been assumed that the multicellular organisms appeared at ~900 to 1000 Ma (*12*).

The Tuanshanzi fossils we have described imply that megascopic multicellular organisms originated at 1700 Ma or earlier. These new data have implications for the understanding of the evolution and other related aspects of Precambrian life. For example, now that the abundance of megascopic metaphytes in the Tuanshanzi Formation is known, the more primitive multicellular and unicellular eukaryotes and even the metazoans may have begun to appear on Earth much earlier than most researchers have estimated (*14*). In addition, theories of the evolution of the Precambrian atmosphere, hydrosphere, and other sedimentary environments that relate to the evolution of Precambrian life must be reexamined in light of the new data.

Table 1. Characteristics of most leaflike megafossils from the Tuanshanzi Formation.

Type	Blades				Stipes	Holdfasts
	Form	Width (mm)	Length (mm)	Length/ width		
1	Spoonlike or tadpolelike	0.4 to 4	5 to 34	6 to 8	Obscure	Discal?
2	Lanceolate	1.2 to 10	6 to 30	3 to 4.3	Ribbonlike	Rhizoidal
3	Elongated lanceolate to ribbonlike	1.3 to 2.6	6 to 20	9 to 10	Obscure	Rhizoidal

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Influence of Sulfide Inhibition of Nitrification on Nitrogen Regeneration in Sediments

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Nitrification, a central process in the nitrogen cycle, converts ammonium to nitrite or nitrate. In experiments with estuarine sediment, addition of 60 and 100 μM hydrogen sulfide (HS^-) reduced nitrification by 50 and 100 percent, respectively. Aerobic incubation of ammonium-enriched sediment slurries showed that previous HS^- exposure reduced nitrification for at least 24 hours; nitrification rates recovered slowly after one-time HS^- exposure. Sulfide inhibition of nitrification could limit nitrogen loss through coupled nitrification-denitrification and may contribute to the previously observed difference in net nitrogen cycling between freshwater and marine sediments. This interaction could also exacerbate eutrophication in coastal environments.

Nitrifying bacteria connect the oxidized and reduced sides of the N cycle by nitrification, the conversion of ammonium to nitrogen oxides. Light, temperature, and substrate concentration affect nitrification, but other factors could also have an effect (*1*, *2*). By serving as a conduit between ammonium regeneration and denitrification, nitrification links N regeneration and N loss (*1*, *2*) (Fig. 1). The relative efficiency of this connection appears to differ between coastal marine or estuarine and freshwater sediments (*3*). Estuarine and marine sediments release similar amounts of ammonium and dinitrogen (N_2), whereas freshwater sediments release mainly N_2 by means of coupled nitrification-denitrification (*4*, *5*). The factor or factors that control this pattern are unclear.

The size of the exchangeable ammonium pool, biogeochemical zonation, and the dominant pathway of metabolism differ between freshwater and marine sediments and could influence the fate of regenerated N (*5*, *6*). In sediments, bacteria oxidize a significant fraction of organic matter using terminal electron acceptors other than oxygen (O_2). Two dominant anaerobic processes are dissimilatory sulfate reduction and methanogenesis. Generally, sulfate reduction (HS^- production) precedes methanogenesis (methane production) because sulfate-reducing bacteria outcompete methanogens for substrates (*6*). Freshwater has lower sulfate concentrations (10 to 200 μM) than does estuarine water (30 mM), and although sulfate reduction may occur in freshwater sediments (*7*), the process is usually less important than methanogenesis.

Because HS^- has been reported to inhibit nitrifying bacteria in biofilm reactors (*8*), we hypothesized that HS^- inhibition of nitrification might account for spatial and temporal differences in nitrification within estuarine environments as well as for the difference in N biogeochemistry between estuarine-marine and freshwater environments (*5*). We tested this hypothesis using sediment from Tomales Bay, California (38.5°N, 122.5°W) (*9*), a small estuary 40 km north of San Francisco. Estuaries and nearshore marine environments serve as centers of deposition for continentally derived organic materials. Most denitrification in marine sediments thus occurs in coastal environments rather than in deep-sea environments, where the sedimentation rate is low (*3*).

Nitrification was rapidly and substantially reduced when 60 or 100 μM HS^- was added to sediment slurries (Fig. 2) (*10*). We found that pulsing with HS^- significantly inhibited nitrification in slurries enriched for nitrifying bacteria, even when the slurries were allowed to recover for 24 hours before we assessed nitrification (Fig. 3) (*11*). The HS^- concentrations we used lie within the range common to estuarine sediments, 7 to 200 μM (*12*), and were much lower than those of organic-rich sediments (>1 mM) (*13*). The range of HS^- concentration in freshwater sediment pore water is much lower (0 to 30 μM) (*12*). In time-series experiments, slurries were amended with HS^- and HS^- plus synthetic goethite, an iron oxide that reacts with HS^-, to determine whether rapid HS^- removal eliminated or reduced the inhibitory effect. Sulfide inhibition persisted despite rapid (≤0.5 hour) removal of HS^- (Fig. 4) (*14*).

Sulfide removal from sediment pore water was likely facilitated by high concentrations of reactive metal oxides (for example, 100 μmol of reactive iron per gram of dry sediment). The availability of reactive iron varies in marine sediments (*15*) and may serve to modulate free HS^- concentration. Despite HS^- addition, the concentration of dissolved O_2 did not differ significantly between HS^--amended and control treatments (Fig. 4). Even though HS^- persisted for only a short time, samples amended with HS^- exhibited significantly lower nitrification rates than did controls (Fig. 4). Thus, only a brief exposure to HS^- was required for inhibition of nitrification.

In the above experiments, the sediment microbial community was exposed to low HS^- concentration in a uniform environment (serum bottles). In situ, nitrification and sulfate reduction are spatially and temporally variable. Both respond to environmental factors, such as bioturbation and burrow irrigation, inputs of organic matter, and benthic primary production, each of which affects the thickness of the aerobic oxidation

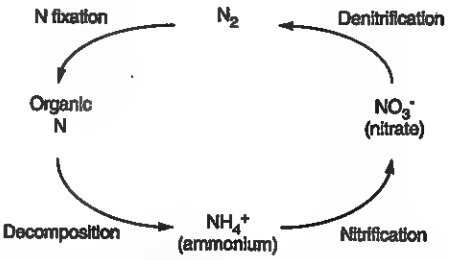

Fig. 1. The transformations of N between gaseous, reduced, and oxidized forms are mediated by bacteria. Gaseous N (N_2) is converted to organic N by N-fixing bacteria. Organic N undergoes decomposition to yield ammonium, which may be assimilated (by bacteria or phytoplankton) or may be nitrified. The processes of nitrification (NH_4^+ oxidation) and denitrification (NO_3^- reduction to N_2) combine to close the nitrogen cycle, returning inorganic, fixed N to the gaseous form. Blocking nitrification or denitrification serves to retain N in a biologically available form (NH_4^+ or NO_3^-).

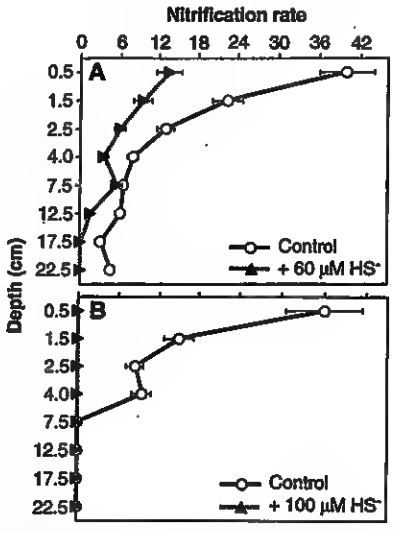

Fig. 2. Effect of HS^- addition [60 μM (**A**); 100 μM (**B**)] on nitrification in estuarine sediments (*10*). Symbols mark the mean of replicate samples (n = 3), and error bars indicate the standard deviation of the mean. Nitrification rate is expressed in nanomoles of NO_x (NO_3 + NO_2) per gram of wet sediment per hour.

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zone in the sediment (*1*, *12*). As a result, the boundary between oxic and anoxic conditions migrates on time scales of seconds to hours and on spatial scales of micrometers to centimeters. Nonmotile nitrifying bacteria are thus exposed to variable conditions that could include periods of HS^- exposure. These processes also vary on seasonal time scales (days to months) and larger spatial scales (meters to kilometers).

Several published studies on N cycling in coastal environments may reflect interactions between HS^- and nitrification. Kemp *et al.* (*16*) showed that during summer in the Chesapeake Bay, sediment nitrification and denitrification reached minima, whereas the benthic N flux reached a maximum. Nitrification increased with bottom water O_2 concentration, which implies that low O_2 concentration limits nitrification. However, this pattern could also reflect HS^- inhibition of nitrification. Under low O_2 conditions, HS^- production will be stimulated while HS^- oxidation is limited. The resulting increase in HS^- concentration could promote inhibition of nitrification by HS^-. Hansen *et al.* (*17*) also observed summer minima in nitrification in Danish coastal sediments. They suggested that HS^- might be an important factor influencing nitrification. Caffrey *et al.* (*18*) showed that nitrification increased with small increases in organic loading but decreased if loading was increased further. They hypothesized that the increase in N regeneration was due to O_2 limitation of nitrification; however, HS^- inhibition also may have been important.

All of these examples of shifts in N regeneration are from estuarine environments. Oxygen concentration also fluctuates in freshwater sediments, but without concomitant production of HS^-. Although O_2 availability might be expected to limit nitrification during summer when benthic metabolism is high, annual maxima in nitrification often occur at this time (*19*). This pattern suggests that O_2 concentration is not the primary variable regulating nitrification in freshwater systems, at least during summer.

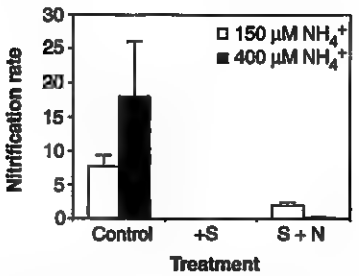

Fig. 3. Effect of HS^- pulses on nitrification in slurries enriched for nitrifying bacteria. No HS^- was present during nitrification assays (*11*). Bars represent the mean nitrification rate (as expressed in Fig. 2) (n = 3), and error bars indicate the standard deviation of the mean. Clear and black bars denote 150 and 400 μM NH_4^+ addition, respectively, during the nitrification assay. Rates in HS^--amended treatments were significantly lower than control rates ($P \leq 0.05$, Fisher's exact test).

Sulfide inhibition of nitrification thus appears to be important in regulating the N cycle of estuarine and marine sediments. It may also explain the pattern of increased N regeneration and less efficient, coupled nitrification-denitrification in estuarine and marine sediments compared with that in freshwater sediments (*5*). Cultural eutrophication or bloom events that increase organic matter sedimentation and stimulate HS^- production in sediments (*20*) could uncouple N regeneration from denitrification by blocking the requisite intermediate step, nitrification. Increased N regeneration could act as a positive feedback loop, enhancing primary production in the water column and accentuating the cycle of cultural eutrophication. For example, increased primary production would stimulate organic matter delivery to sediments, which would stimulate organic matter oxidation and sulfate reduction, leading to higher HS^- concentrations and HS^- inhibition of nitrification. Sulfide inhibition of nitrification would increase benthic N regeneration; higher ammonium fluxes to the water column could further stimulate primary production. Denitrification is also inhibited by HS^- (*21*); thus, the effect of HS^- on N cycling in marine sediments includes both portions of the N-sink couple. Other reduced S compounds, such as polysulfides, which may have a longer half-life than does HS^-, may also inhibit nitrification.

The seasonality of sulfate reduction, HS^- production, and HS^- inhibition of nitrification and denitrification should lead to enhanced ammonium regeneration during summer, when sulfate reduction rates are high compared with those in winter (*12*). Such seasonal or episodic enhancement of ammonium regeneration could result in shifts in the nutrient (N, P, or Si) limiting primary production (*22*). Internal control of the sedimentary N cycle by HS^- appears to be an important factor regulating the fate of sediment N and demonstrates a link between the global biogeochemical cycles of N and S.

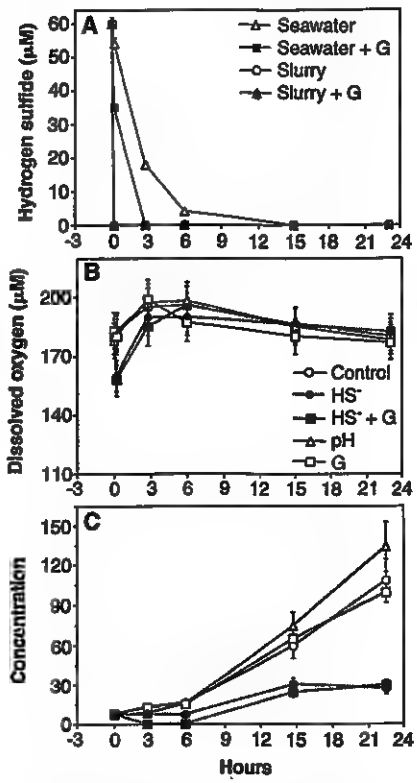

Fig. 4. Concentration of HS^- (**A**), dissolved O_2 (**B**), and NO_x accumulation (**C**) in sediment slurries (*12*). For (A), data from seawater only (seawater) and seawater plus sediment (slurry) treatments, with or without goethite, are shown. Symbols represent the mean (n = 3); error bars denote the standard deviation of the mean. Nitrification rates were significantly lower (analysis of variance, $P < 0.05$) in HS^--amended compared with control treatments. Concentration in (C) is a function of nanomoles of NO_x per gram of wet sediment. Symbols in (C) are the same as those in (B).

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10. Two sediment cores (30 cm) were collected (January 1994) and sectioned into discrete depth intervals; the salinity of the bay was 30 practical salinity units. Samples from each depth were slurried [1.5 g of wet sediment + 50 ml of filtered (glass fiber filter, 0.7 μm nominal pore size) seawater] and placed in 70-ml serum bottles. Bottles were amended with ammonium (NH_4^+, 300 μM) or NH_4^+ plus 60 or 100 μM HS^- and sealed. Samples were incubated in the dark with constant shaking (100 rpm). Replicates were collected and analyzed at intervals between 0 to 24 hours to ensure linearity in nitrification rates [linear regressions of ($NO_3^- + NO_2^-$) versus time had r^2 values of ≥0.98]. Samples did not become anoxic during the incubation (see Fig. 4). After incubation, a 15-ml aliquot was filtered (GF/F) and immediately frozen for subsequent NO_x and NH_4^+ analyses. [NO_x] was determined with the spongy cadmium reduction method (*23*), and nitrification rates were calculated from the change in NO_x concentration over time.
11. Surface (top 5 cm) sediment was sieved (1-mm

mesh) and slurried with filtered bottom water. Three sterile flasks each received 500 ml of sediment slurry. The three flasks were as follows: control, amended with 400 μM NH_4^+; +S, pulsed with 100 μM HS^- twice daily; or S + N, amended with 400 μM NH_4^+ and pulsed with 100 μM HS^- twice daily. During enrichment, hydrated air flowed continuously through the cultures. Approximately 24 hours after the last HS^- pulse was administered, nitrification rates were assessed in subsamples amended with either 150 or 400 μM NH_4^+.

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14. Surface sediments (0 to 3 cm) were sieved, homogenized, and randomly distributed [1 gws (gram of wet sediment)] into 40-ml serum bottles. Thirty milliliters of filtered seawater, amended with 300 μM NH_4^+, was added to each bottle; 18 replicates were prepared for each treatment group. Treatment groups were as follows: control, NH_4^+ only; pH, pH adjusted with NaOH to match that in HS^--amended samples; goethite (G), amended with 700 μM goethite; HS^-, amended with 60 μM HS^-; and HS^- + G, amended with 60 μM HS^-, then with 700 μM goethite. Triplicate samples from each group were collected and analyzed at approximately 0.5, 3, 6, 12, and 23 hours. Sulfide was determined as in (*24*). We quantified oxygen concentration by inserting microelectrodes (Diamond General, Ann Arbor, MI) into bottles before collecting nutrient samples. Oxygen was not limiting during these experiments. NO_x concentration was determined as described previously (*10*).
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Cross-Arc Geochemical Variations in the Kurile Arc as a Function of Slab Depth

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Lavas from transects across the Kurile Islands arc showed geochemical variations related to changes in the compositions of fluids derived from the subducting slab. Enrichment factors for boron, cesium, arsenic, and antimony, all elements with strong affinities for water, decreased across the arc. This decrease is presumably related to losses of water-rich fluids during the dehydration of the subducting plate. Enrichments of potassium, barium, beryllium-10, and the light rare earth elements remained constant; these species may move in silica-rich fluids liberated from the slab at greater depths.

The involvement of slab-derived melts (*1*, *2*) and the importance of nonmagmatic (that is, fluid) slab components (*3*–*6*) in arc magmatism are uncertain and much debated. Only recently have clear indicators for slab contributions been identified: High concentrations of B and ^{10}Be in arc lavas require material inputs to arc source regions from subducted oceanic sediments and altered oceanic crust (*4*, *7*). To better understand magma generation at arcs and the significance of subduction in crustal recycling, we need to know what processes control material fluxes from the subducting plate and how these fluxes interact with the overlying mantle.

We examined trace element systematics in suites of lavas from a series of "cross-arc transects" across the arc of the Kurile Islands. The volcanoes sampled lie 120 to 250 km above the subducting plate and may thus reflect slab-mantle interactions occurring over a range of pressure and temperature conditions. The Kurile Islands are a stereotypic island arc, erupting medium K calc-alkaline lavas across an unusually wide volcanic zone (Table 1) (*8*). Most Kurile lavas that have been analyzed have ^{10}Be contents greater than 1.0×10^6 atoms/g, which indicates subducted sediment (slab) involvement during magma genesis. Limited isotopic variation ($^{87/86}Sr \approx 0.703$ to 0.7034 and $^{143/144}Nd \approx 0.5130$ to 0.5131) (*9*) suggests that neither enriched mantle sources nor crustal assimilation strongly affects lava chemistry.

Data for six Kurile Islands cross-arc transects are presented in Table 1 and Fig. 1 (*10*). Incompatible trace elements show a spectrum of across-arc variation patterns. B and Sb (*11*) reflect one extreme (Fig. 1A)—high concentrations in volcanic front (VF) lavas, with abundances declining across the arc. Cs and Rb (Fig. 1C) show no clear pattern of concentration variation, whereas K, Ba, and most other elements (Fig. 1, E and G) increase in concentration across the arc. These element abundance patterns result from slab inputs, which may vary with increasing slab depth, and from varying extents of partial melting and crystal fractionation. We disentangled these effects by determining the ratios of the elements of interest to other elements with similar solid-melt distribution coefficients (*D*s) but much lower apparent solid "slab fluid" *D*s. Use of these element ratios minimized the effects of processes other than subduction modification of the mantle. K_2O, La, and B all have solid or melt *D*s similar to that of Be; the solid or melt *D* for Cs is more similar to that of Th. Thus, in

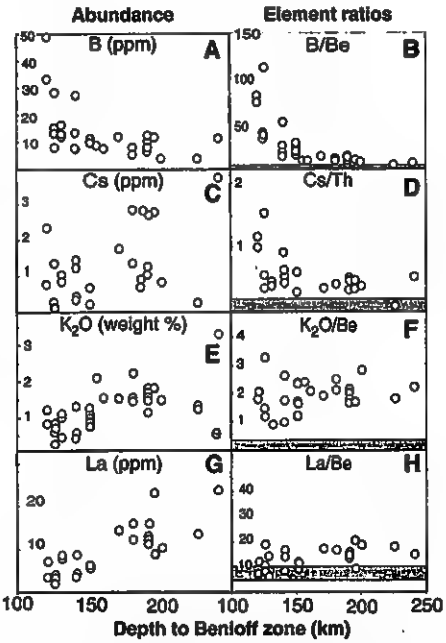

Fig. 1. Plots of element abundance and ratios for cross-arc transects in the Kurile Islands. Depths to Benioff zone were determined with the use of data from (*32*) and (*33*); absolute uncertainties in depths are ±10 km, but volcano-to-volcano uncertainties are much smaller. Gray fields on ratio diagrams represent ranges for mid-ocean ridge basalt and Ocean island basalt sources based on (*5*) and (*35*) and references therein. (**A** and **B**) B and B/Be versus depth. (**C** and **D**) Cs and Cs/Th versus depth. (**E** and **F**) K_2O and K_2O/Be versus depth. (**G** and **H**) La and La/Be versus depth.

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Fig. 1, B/Be, Cs/Th, K_2O/Be, and La/Be ratios are plotted versus depth.

It can be demonstrated that lavas erupting at the rear of an arc are produced by generally lower degrees of partial melting than are those at the VF (*12*). This effect will produce positive slopes on abundance diagrams, both when no slab-related elemental inputs are evident and when inputs are constant across the arc (Fig. 2A). In either case, ratio diagrams (Fig. 2B) show subhorizontal trends versus slab depth, with trend positions shifted to values higher than those of the normal upper mantle if inputs from the slab have occurred. K and Ba behave as if approximately constant subduction additions occur across the arc, whereas La shows only slight enrichments relative to upper mantle values.

As a highly incompatible element, B should show patterns similar to those in Figs. 2A or 2B, unless its abundance in the mantle decreases dramatically enough across the arc to mask the effects on B concentration of lower degrees of melting behind the front (Fig. 2, C and D). B/Be ratios decline from as high as 125 at the VF to <10 in the rearmost volcanoes, which suggests that inventories of slab-derived B in the mantle decrease across the arc (Fig. 1B). Cs/Th ratios (Fig. 1D) show less pronounced but significant across-arc declines, which indicates that mantle wedge Cs inventories show comparable changes. The patterns of B/Be and Cs/Th variation across the Kurile Islands resemble changes observed in subduction-related metamorphic massifs such as the Catalina Schist (*13*, *14*), where reductions in B and Cs concentrations as grade increases correlate with decreases in N and H_2O concentrations.

The Kurile Islands results demonstrate that slab signatures extend beyond VF source regions beneath arcs, and that transport of these signatures into behind-the-front (BTF) sources produces specific elemental fractionations. Mechanisms for slab-mantle chemical exchange beneath arcs must explain the differing trace element variation patterns observed across the Kuriles. Inputs of slab sediment melts will explain enrichments of K, Ba, Sr, and La in arc lavas (*2*), but cannot reconcile across-arc changes in the abundances of similarly incompatible elements such as B. Metamorphic devolatilization processes progressively remove B and Cs from the slab sediments beginning at relatively low grades (*13–17*), but do not substantially mobilize K or Ba over the same range of pressure and temperature. Fluids derived from metamorphosing oceanic crust have been proposed to balance the Sr and Pb budgets of arcs (*18*) and to explain B isotope systematics across arcs (*19*). Such fluids could both trigger sediment melting and enrich the melts in fluid-soluble species. However, B and Cs abundances in old ocean crust are much lower than in trench sediments (*2*, *20*). Also, the high ^{10}Be concentrations and $^{10}Be/^{9}Be$ ratios in Kurile Islands lavas require slab inputs in which 100% of the ^{10}Be is provided by subducted sediments (*4*). Thus, it is likely that large amounts of B and of most other trace elements added to arc source regions are derived from subducted sediments, the origins of slab fluids notwithstanding.

Current models of subduction zone chemical exchanges envision the slab input process as a point-source event, with the slab signature transported either downdip in hydrated mantle near the slab (*21*) or subhorizontally through the wedge to magmatic source regions (*22*). The horizontal trans-

Table 1. Selected elemental data for Kurile Islands transects. Dashes indicate no analysis.

Sample	Slab depth	SiO_2 (weight %)	MgO (weight %)	K_2O (weight %)	B (ppm)	Be (ppm)	Ba (ppm)	Cs (ppm)	La (ppm)	Th (ppm)	Sb (ppm)	As (ppm)
North Iturup transect (45° to 46°N)												
B-15-392	125	57.4	5.51	0.77	29.0	0.23	191	1.4	4.5	0.9	*0.44*	*4.7*
B-15-394	125	51.0	3.63	0.27	8.6	0.22	86	0.18	2.5	0.3	*0.11*	*1.2*
B-15-81/1	170	54.3	4.77	1.66	12.9	0.84	378	1.8	14.9	4.6	*0.10*	*0.78*
B-15-80/5	190	64.6	1.37	1.22	8.6	0.71	281	1.1	11.8	3.0	*0.07*	*0.48*
B-15-73/1	240	60.6	3.51	3.56	12.2	1.55	629	3.8	24.3	6.6	*0.10*	*0.88*
South Iturup transect (44° to 45°N)												
B17-643	125	49.1	4.80	0.15	5.1	0.19	275	0.56	1.1	0.2	–	–
B17-642	125	59.6	3.11	0.63	13.8	0.41	365	0.34	3.7	0.9	0.17	2.4
B-15-356	125	65.7	1.38	0.88	16.0	0.52	242	–	–	–	–	–
B17-640	140	50.2	4.25	0.46	8.7	0.45	319	0.41	4.1	0.4	–	–
B17-34/1	195	57.8	3.88	1.95	12.4	1.11	844	2.9	23.4	7.6	0.07	0.90
Chirpoy transect (46°30'N)												
B-15-325	140	60.7	3.10	1.42	28.0	0.53	237	1.5	9.2	2.6	*0.4*	*5.1*
B-17-684	140	51.1	5.05	0.60	8.2	0.33	138	0.47	4.9	1.0	*0.08*	*0.92*
B-17-603	225	52.3	7.70	1.40	4.5	0.75	341	0.31	14.0	2.7	*0.05*	*0.44*
Chirinkotan transect (48° to 49°N)												
B-11-112/9	130	55.3	4.27	1.06	12.6	0.52	246	1.1	7.9	2.4	0.07	1.7
B-11-111/7	130	56.8	3.67	0.48	16.9	0.42	137	–	–	–	–	–
B-11-111/3	130	59.4	3.52	1.14	12.9	1.26	276	0.80	8.4	2.1	0.17	0.95
B-11-569	150	56.3	4.13	1.05	10.3	0.43	239	–	–	–	–	–
B-11-570	150	58.5	4.00	1.25	14.7	0.51	432	1.2	6.2	1.7	0.09	2.0
B-11-527	190	56.3	2.80	1.95	13.0	0.89	638	2.7	8.4	4.9	–	0.89
Onekotan transect (49°15' to 50°N)												
H-3	120	61.5	2.08	0.88	34.1	0.46	269	0.82	3.8	0.8	*0.54*	*5.7*
8322/3	120	64.7	1.93	1.31	49.6	0.61	307	2.4	7.7	2.0	*0.70*	*9.2*
B11-81/4	155	60.1	3.19	2.29	9.4	0.93	725	2.3	14.1	5.1	–	1.3
B-11-504	160	56.6	4.74	1.67	8.4	0.77	436	–	–	–	–	–
B-11-75/4	180	52.2	4.47	1.58	8.9	0.73	399	1.4	12.7	3.0	*0.09*	*0.87*
B-11-73/13	180	62.4	2.41	2.43	–	0.96	657	2.9	16.3	6.5	0.11	2.0
B11-74/1	180	50.9	5.21	1.65	5.9	0.64	499	0.91	10.5	2.3	–	–
B-11-72/3	200	48.0	5.24	1.60	4.6	0.55	359	0.89	10.8	2.1	*0.04*	*0.57*
Paramushir-Alaid transect (50°30' to 51°N)												
4/2 81	150	58.4	3.40	1.17	10.7	0.68	420	–	–	–	–	–
B25-865	150	58.4	2.54	0.82	11.4	0.67	396	0.24	6.5	0.7	0.05	1.3
PARA 16-1	150	53.0	3.97	0.86	11.8	0.50	385	0.73	6.2	1.1	0.09	1.8
B-11-575	190	50.4	4.41	1.79	9.7	0.88	341	1.2	12.3	2.2	–	–
2/381	190	51.2	3.82	1.60	7.9	0.88	332	–	–	–	–	–
B-11-576	190	51.0	4.23	1.58	11.0	0.85	313	–	–	–	–	–
K-8	190	51.3	4.03	1.84	13.0	0.88	372	1.3	13.4	2.5	0.30	–
B-11-52/1	225	47.2	5.07	1.38	–	–	221	–	–	–	–	–
B-11-52/2	225	47.8	6.44	1.33	–	–	235	–	–	–	–	–

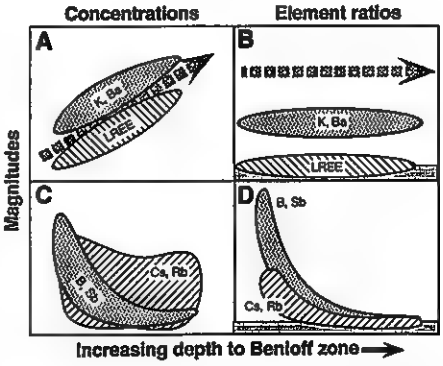

Fig. 2. Schematic illustration of concentration and ratio systematics. Fields depict lava concentration trends; arrows show trajectories produced by progressive variations in extents of melting or crystallization. LREE are the light rare earth elements. (**A** and **B**) Concentration and ratio patterns versus depth for trace elements, which show uniform or minimal inputs from the subducting plate over a range of depths. (**C** and **D**) Content and ratio patterns for elements such as B, in which inputs from the subducting plate vary as a function of slab depth. Gray regions in (B) and (D) represent ratio values for oceanic mantle sources.

port scenario requires that phases hosting slab-derived species survive melting events at the VF (and one or more BTF melting events) and that the observed elemental fractionations be generated largely through mineral-melt equilibria. However, many phases proposed to host slab fluids in the wedge [for example, antigorite, lawsonite, and "phase A" (*23*, *24*)] decompose well below sub-arc melting temperatures. Amphibole and phlogopite are stable at higher temperatures but will be preferentially consumed during melting. Transport of fluids in the absence of hydrous phases (that is, as H_2O dissolved in "dry" mantle minerals) also seems unlikely, as the solid-melt distribution behavior of H_2O is similar to that of Ce (*25*). Arc melting would thus extract H_2O, K, B, and other incompatibles from the mantle with similar efficiency, precluding substantial across-arc fractionations.

Transport of the slab signature in hydrated mantle near the slab circumvents thermal stability problems, but requires that partitioning between solids and fluids during mineral decompositions generate across-arc elemental fractionations. Experimental results on antigorite decomposition suggest an inverse correlation between the ionic radius and the solid-fluid partition coefficient for alkaline elements (*23*). However, new data for subduction-related serpentines suggest that antigorite may be a poor host for Ba and K (*26*). Lawsonite hosts Sr well but hosts other alkaline elements only poorly; amphiboles and phlogopites, however, may retain inventories of K, Ba, and other alkaline species (*24*, *27*). B partitions strongly into serpentines (*26*) and may partition modestly into lawsonite (*24*), but B partitioning affinities into amphibole or phlogopite (and the partitioning of Sb or As into any of the above phases) are unknown. To generate the across-arc changes in B and like species, however, the solid-fluid partition coefficients of these elements during mantle dehydration must be <1, so that slab-derived inventories are depleted with depth. An added constraint on all mantle transport models relates to observed ^{10}Be variations (*28*): Transport times from the VF to the rearmost centers in the Kurile Islands cannot exceed one ^{10}Be half-life. The slab signature must therefore travel into BTF source regions at a velocity comparable to that of the slab.

Implicit in the above models is the presumption that the slab dehydrates at depths too shallow to generate arc lavas. If, however, the slab can retain fluids to greater depths, complications such as the ^{10}Be timing constraint disappear. As mentioned above, B, Cs, N, and H_2O all show regular abundance declines with increasing pressures and temperatures in metamorphic massifs believed to represent emplaced portions of slabs. All of these species, and most other alkaline elements, are predominantly hosted by phengitic white micas (*24*). White mica persists over a large range of pressure and temperature conditions during metamorphism and may be stable to <200 km on the slab (*29*). Fluid compositions during slab dehydration would thus be controlled by white mica re-equilibrations with increasing depth, resulting in the liberation of B, Sb, and Cs (but not of K, Ba, or La) in hydrous fluids.

For an on-slab transport model to produce the across-arc elemental patterns of the Kurile Islands, a mechanism must also exist to mobilize elements such as K and La [and Be (*4*, *12*)], which appear not to partition strongly into fluids that transport B and Cs. Melts of slab sediments in response to mica dehydration are capable of mobilizing these species in appropriate relative proportions (*2*, *30*). It is thus possible that a mixed slab flux, composed of both hydrous fluids and sediment melts, is input to the mantle beneath the Kurile Islands and other arcs. High-grade rocks of the Catalina schist include both veins and pegmatites, which suggests that liberating two compositionally different "fluids" from the slab under the same pressure and temperature conditions is possible (*31*). Variations in element ratios among VF lavas may thus relate to differing proportions of fluid and melt inputs to VF sources, whereas BTF magmas may record the "drying out" of the slab as hydrous fluids (with B, Sb, and Cs) are driven off with increasing depth. Because all Kurile Islands lavas show similar K/Be and Ba/Be ratios, all source regions across the arc must receive inputs of compositionally similar melts. Minimum VF slab temperatures may thus be approximately equivalent to those encountered during amphibolite grade metamorphism. Sufficient B can persist on the slab at these temperatures to generate the elevated B/Be ratios observed in VF lavas from the Kurile Islands and other arcs (*13*, *17*). Further metamorphism with deeper subduction will drive off the remaining inventories of B and like elements, producing their depleting across-arc variation patterns. But melt inputs, even if reduced in magnitude in response to dehydration at depth, should maintain trace element signatures reflecting equilibration with slab residues.

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A Methylnickel Intermediate in a Bimetallic Mechanism of Acetyl–Coenzyme A Synthesis by Anaerobic Bacteria

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Resonance Raman (RR) spectroscopy was used to identify a methylnickel adduct (ν_{Ni-C} = 422 wave numbers) of carbon monoxide dehydrogenase (CODH) from *Clostridium thermoaceticum*. Formed at a nickel/iron-sulfur cluster on CODH called center A, the methylnickel species is the precursor of the methyl group of acetyl–coenzyme A in an anaerobic pathway of carbon monoxide or carbon dioxide fixation. Rapid kinetic and RR studies demonstrated that methylation of nickel occurs by heterolysis of the methyl-cobalt bond (ν_{Co-C} = 429 wave numbers) of a methylated corrinoid/iron-sulfur protein. In combination with the earlier finding of an iron-carbonyl adduct at center A, detection of the methylnickel intermediate establishes a bimetallic mechanism for acetyl–coenzyme A synthesis.

Carbon monoxide dehydrogenase is a metalloenzyme that catalyzes the final steps in the reductive acetyl–coenzyme A (acetyl-CoA) pathway. This pathway allows anaerobic bacteria to grow on CO_2 or CO as a sole carbon source and methanogenic archae to convert acetic acid to methane. The final steps involve activating and combining a methyl group and CO to form an acetyl group which is then incorporated into acetyl-CoA. Here we establish that the C-C bond is formed by an organometallic mechanism.

CODH contains 2 Ni, 11 to 14 Fe, ~1 Zn, and ~14 inorganic sulfides per heterodimeric unit (*1*). The metals are organized into three clusters, centers A, B, and C (*2*). Centers A and C (*3*, *4*) are Ni-FeS clusters. Center A catalyzes the final steps in acetyl-CoA synthesis (*2*, *5*, *6*). A minimal structure for center A has been defined by spectroscopic studies to be Ni-X-[4Fe-4S], where X is an unknown ligand bridge (*7*). An adduct between CO and center A was shown to be kinetically competent as the precursor of the carbonyl group of acetyl-CoA (*2*, *5*). Surprisingly, this adduct was found to be a complex between CO and Fe, not Ni (*3*, *8*, *9*). What, then, is the role of nickel, which is required for acetyl-CoA synthesis (*6*, *10*)? A bimetallic mechanism for acetyl-CoA synthesis was proposed that included iron-carbonyl and methylnickel intermediates (*9*).

The methyl group of acetyl-CoA is derived from CO_2 through steps that involve formate dehydrogenase, a series of tetrahydrofolate (H_4folate) enzymes, and a corrinoid/iron-sulfur protein (corrinoid-FeS protein), forming a methylcobamide species on the corrinoid-FeS protein (*11*). This methylcobalt species donates the methyl group to CODH. Our first goal was to characterize the methylcobalt bond. We methylated the corrinoid-FeS protein (*12*) with CH_3I and identified the methyl-Co stretching band at 429 cm^{-1} in the resonance Raman (RR) spectrum (Fig. 1). This band moved to 420 cm^{-1} when the corrinoid-FeS protein was methylated with $^{13}CH_3I$ (*13*). The band position indicates that the Co-C bond is weaker and longer than that of free methylcobalamin in solution and other six-coordinate organocobalt B_{12} model compounds that exhibit Co-C stretching modes at ~500 cm^{-1} (*14*).

Although it has been presumed that methylation of CODH occurs at center A (*15*), direct evidence has been lacking. When CODH (*16*) was reacted with the $^{12}CH_3$-corrinoid-FeS protein (*17*), the 429-cm^{-1} band from methyl-Co diminished and a new band at 422 cm^{-1} appeared (Fig. 2). This band moved to 410 cm^{-1} or to 392 cm^{-1} when $^{13}CH_3$-corrinoid-FeS protein or CD_3-corrinoid-FeS protein was the methyl donor (*18*) . Therefore, this band was assigned as the stretching frequency for the methylmetal bond at center A of CODH. The 422-cm^{-1} band was also observed when CODH was reacted with $^{12}CH_3I$ and catalytic amounts of the corrinoid-FeS protein (*19*).

To identify the methyl acceptor, we isolated CODH from cells grown in medium containing ^{54}Fe (98%), ^{58}Fe (85%), or ^{64}Ni (95%). (Natural abundance masses are 55.9 for Fe and 58.7 for Ni.) When ^{54}Fe or ^{58}Fe was substituted into CODH, the metal-CH_3 stretching band remained at 422 cm^{-1}, the position observed with ^{56}Fe-CODH (Fig. 2). Therefore, the methyl group does not bind to an iron site in center A. However, when ^{64}Ni-CODH was methylated with the methylated corrinoid-FeS protein, the band moved to 417 cm^{-1}, establishing the mode as a Ni-methyl stretch (*20*).

These results demonstrate that Ni accepts the methyl group from the methylated corrinoid-FeS protein. The transfer reaction could involve homolytic or heterolytic cleavage of the methyl-Co^{3+} bond. These two mechanisms were distinguished by determining whether the transmethylation reaction generated Co^{2+} or Co^{+} as the prod-

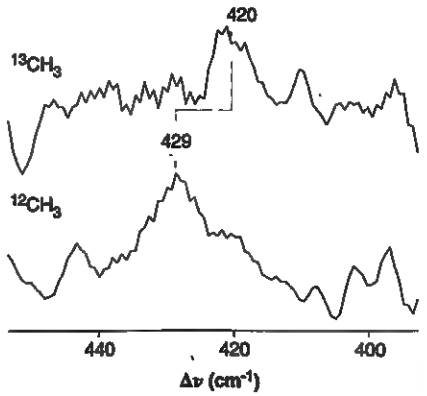

Fig. 1. RR spectra at 77 K of methylated corrinoid-FeS protein, methylated with $^{12}CH_3I$ (bottom) and $^{13}CH_3I$ (top). The scattered light from a 476.5-nm Ar^+ ion laser line (~70 mW) was focused into a triple monochromator equipped with a diode array multichannel detector. The collection time was ~3 hours per spectrum. Spectra were calibrated with carbon tetrachloride and dimethyl formamide.

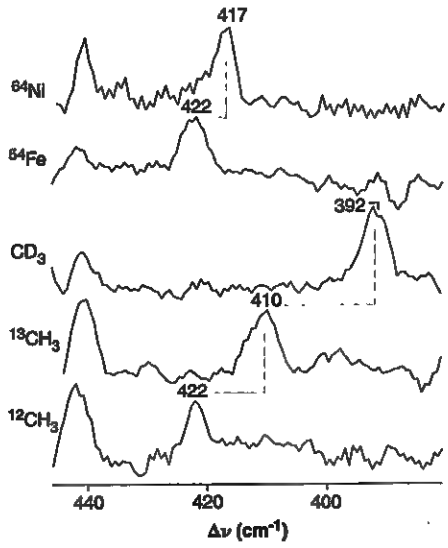

Fig. 2. RR spectra (476.5 nm–excited, 77 K) of the indicated isotopomers of reduced CODH treated with methylated corrinoid/Fe-SP (corrinoid-FeS protein spectrum subtracted digitally). The top two spectra show the effect on the 422-cm^{-1} band (metal-CH_3 stretching) of labeling CODH with ^{64}Ni and ^{54}Fe, whereas the bottom three spectra show the effect of increasing the mass of the methyl group. Conditions as in Fig. 1.

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uct. The product of a heterolytic cleavage would be Co^+, characterized by a sharp absorption peak at 390 nm [$\Delta\epsilon$ (extinction coefficient change) = 5 mM^{-1} cm^{-1}]. The product of homolytic fission would be Co^{2+}. Co^{2+} in the corrinoid/FeS protein adopts a four-coordinate geometry in which the benzimidazole base is displaced (termed base-off) resulting in a broad absorption band centered at 470 nm (*21*). A Co^+ product was established by stopped-flow kinetics (*22*). When CODH was rapidly mixed with CH_3-Co^{3+}-corrinoid-FeS protein, Co^+ was formed (Fig. 3). Clean isobestic points were observed at the expected wavelengths, indicating that methyl-Co^{3+} was converted to Co^+ without an intermediate. That Co^+ is the product of the transmethylation reaction is supported by single wavelength–monitored kinetics (*23*). The decay rate for the 450-nm peak of base-off methyl-Co^{3+} was equivalent to the formation rate for the 390-nm peak that characterizes Co^+ (1.2 s^{-1} at 25°C) (Fig. 3, inset). Extrapolating this rate constant to 55°C, the optimal growth temperature for *Clostridium thermoaceticum*, gave a rate constant of ~10 s^{-1} (*24*). Thus, formation of Co^+ is catalytically relevant because it occurred approximately fivefold faster than the k_{cat} for acetyl-CoA synthesis (*25*). Generation of Co^+ would be advantageous for the cell because it can then react with CH_3-H_4 folate to regenerate the methylated corrinoid-FeS protein. For a homolytic radical mechanism, a one-electron reduction of Co^{2+} would be required with each cycle of acetyl-CoA synthesis.

Previous studies on the corrinoid-FeS protein indicate that it is designed to facilitate heterolytic Co-C bond cleavage. The cobamide is base-off in all three oxidation states (*21*, *26*). Absence of a nitrogenous donor ligand in the methyl-Co(III) state has been reported to predispose the Co-C bond toward heterolytic cleavage and protect against radical chemistry (*27*). Thus, it is possible that the corrinoid-FeS protein modulates the cobalt coordination chemistry to activate the methyl group toward a nucleophilic attack. Heterolytic cleavage of the methyl-Co bond to generate Co^+ as the product requires the participation of a nucleophilic group on CODH.

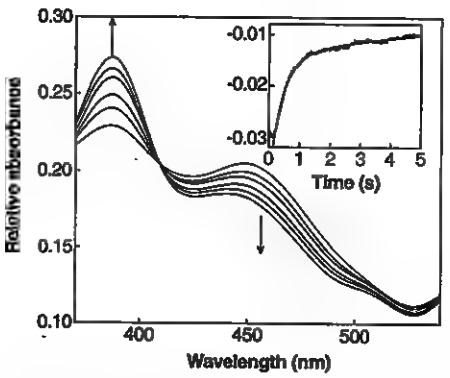

Fig. 3. Reaction of reduced CODH with methylated corrinoid/Fe-SP. CODH was incubated with CO for 15 min and mixed rapidly with the methylated corrinoid/FeS protein. The reaction was repeated as the monochromator was moved in 10-nm increments between 370 and 520 nm. Time slices were obtained by digitally connecting the data points 10 nm apart, using the "point-by-point" software provided by Applied Photophysics. (**Inset**) Single wavelength–monitored kinetics of the reaction of 10 μM CODH with 10 μM methylated corrinoid/FeS protein followed at 390 nm.

The bio-organometallic catalytic cycle catalyzed by CODH can be summarized [see figure 3 of (*9*) or figure 1 of (*28*)] as follows: (i) An Fe-CO species is formed at center A (*29*). The CO originates from the atmosphere or from the reduction of CO_2 to CO by center C of CODH. (ii) The low-valent Ni site in center A performs a nucleophilic attack on the methylated corrinoid-FeS protein, generating Co^+ and methylnickel. (iii) The next step in acetyl-CoA synthesis involves either a carbonyl insertion to form acetyl-Ni or a methyl migration to generate an acetyl-Fe intermediate. (iv) The final step involves thiolysis of the acetyl-metal intermediate to form acetyl-CoA. A suggested mechanism for this step involves a metal-SCoA intermediate (*28*).

We have demonstrated a bimetallic enzymatic mechanism of C-C bond formation. There are several examples of bimetallic catalysts in the organometallic chemical literature. A heterobimetallic complex containing CH_3-Zr and Mo-CO undergoes further carbonylation to form an acetyl complex (*30*). Reaction of methyl-$Mn(CO)_5$ with an Fe-carbonyl complex yields a heterobimetallic bridging acetyl complex (*31*). In addition, there are examples of heterolytic (*32*) and homolytic cleavage of an alkylmetal complex by another metal (*33*). Examples of CO insertion and methyl migration reactions have been reviewed (*34*). Another example is the "Monsanto process" for acetic acid synthesis from methanol and CO in which a rhodium or iridium catalyst undergoes methylation, carbonylation, and methyl migration to form acetyl-rhodium (*35*).

Nickel is an essential trace element for bacteria, plants, animals, and humans (*36*). It is an essential component of four enzymes (CODH, urease, methyl-CoM reductase, and hydrogenase). Our results provide convincing direct evidence for a new biological role of nickel. The methylnickel intermediate represents the first example of a Ni-C bond in nature. Adenosylcobalamin and methylcobalamin are the only other known examples of alkylmetal bonds in enzymes (*37*). Demonstration of a methylnickel intermediate in acetyl-CoA synthesis sets a biological precedent relevant to the mechanism of methane synthesis from methyl-CoM. It has been suggested that this reaction could occur through a methylnickel intermediate (*38*).

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12. The corrinoid-FeS protein was purified to homogeneity as described (*39*). Methylation of the corrinoid-FeS protein [46 mg in 1 ml of 50 mM tris-HCl (pH 7.6)] was achieved by first reducing it with Ti(III) citrate (5 mM, final) for 1 hour, adding 1 μl (16 μmol) of neat CH_3I, and incubating at 16°C for 30 min. The methylated corrinoid-FeS protein was then centrifuged through a Sephadex G-25 column to remove unreacted CH_3I and Ti. The methylated corrinoid-FeS protein was concentrated with an Amicon macrosolute concentrator. By evaluating this reaction with tracer amounts of $^{14}CH_3$-I, we found that the corrinoid-FeS protein was 96% methylated. Earlier studies had demonstrated that CH_3I can be used in the synthesis of acetyl-CoA and that the methylated corrinoid-FeS protein generated by reaction with CH_3I is catalytically active (*39*, *40*).
13. If the $^{13}CH_3$ and $^{12}CH_3$ groups are treated as point masses of 16 and 15 atomic mass units (amu), respectively, the calculated frequency ratio of the Co-$^{13}CH_3$ and Co-$^{12}CH_3$ stretching bands would be 0.975, which matches well with the observed value of 0.979.
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16. CODH was purified as described (*1*) to a specific activity measured at 55°C of 521 U/mg in the CO oxidation reaction and 0.960 U/mg in the exchange reaction between CO and [1-^{14}C]acetyl-CoA. One

unit is defined as 1 μmol of CO exchanged per min.

17. CODH [120 to 140 mg ml^{-1} in 50 mM tris-HCl (pH 7.6)] was reduced with CO (1 atm for 10 to 15 min) or with dithionite (4 mM final) and then methylated by adding the methylated corrinoid-FeS protein (1.5 to 2 equivalents) with vigorous shaking. The reaction mixture was placed in the RR sample holder and frozen cryogenically within 2 to 4 min after mixing the two proteins.
18. Treating the $^{13}CH_3$ and $^{12}CH_3$ groups as point masses of 16 and 15 amu, respectively, the calculated frequency ratio of the Ni-$^{13}CH_3$ and Ni-$^{12}CH_3$ stretching bands would be 0.975, which matches well with the observed value of 0.969. The calculated frequency ratio of the Ni-CD_3 and Ni-CH_3 stretching bands is 0.931, in agreement with the observed value of 0.927.
19. We also used CH_3I as the methyl group donor to CODH. This reaction requires the intermediacy of the corrinoid-FeS protein, because the methylated corrinoid-FeS protein is the only known direct methyl donor for CODH (*40*). CODH [40 nmol, 6 mg, in 100 μl of 50 mM tris-HCl (pH 7.6)] was mixed with 0.40 nmol of corrinoid-FeS protein under a CO atmosphere for 15 min. The atmosphere was exchanged with nitrogen, 4 μmol of CH_3I (20 μl of 0.2 M) was added, and the solution was incubated for 30 min. Excess CH_3I was removed by centrifuging the mixture through a Sephadex G-50 column, and the protein was concentrated with Amicon centricon tubes. By monitoring with tracer amounts of $^{14}CH_3I$, it was shown that 0.89 mol of methyl groups were incorporated per mol of CODH. When $^{13}CH_3I$ was the methyl donor, the methyl-metal band was observed at 410 cm^{-1}.
20. The calculated frequency ratio of the ^{64}Ni-$^{12}CH_3$ and ^{58}Ni-$^{12}CH_3$ stretching bands would be 0.990, which matches well with the observed value of 0.988. If an Fe-CH_3 bond had been observed, the position of the ^{58}Fe-CH_3 band would be expected to shift by 4 cm^{-1} relative to the ^{54}Fe-CH_3 sample. Natural abundance Ni consists of ^{58}Ni and ^{60}Ni in ~2:1 ratio. The sharpness of the 417-cm^{-1} band is interpreted to reflect the presence of a single Ni isotope. This provides further evidence that Ni is involved in this resonance.
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22. Stopped-flow experiments were performed on an Applied Photophysics spectrofluorimeter and data were fit with software purchased from Applied Photophysics. Solutions of enzymes and substrates were made in the anaerobic chamber, transferred into tonometers that are isolated from the atmosphere by stopcocks, and connected directly to the drive syringes of the stopped-flow instrument. These syringes were maintained anaerobically in a temperature-controlled bath of anaerobic water.
23. The possibility was ruled out that Co^{2+} is the product of methyl transfer but is rapidly reduced to Co^{+} by CODH in a subsequent step. In a separate experiment with the Co^{2+}-corrinoid-FeS protein (5 μM) and CO-reduced CODH (2.5 μM), the k_{obs} for Co^{2+} reduction was 0.026 ± 0.003 s^{-1} at 30°C and 0.037 ± 0.003 s^{-1} at 40°C. These rates are significantly lower than the rate of formation of Co^{+} in the methyl transfer reaction. We and others earlier ruled out a mechanism involving one-electron reduction of methyl-Co^{3+} followed by homolytic cleavage, which would also generate Co^{+} [B. D. Martin and R. G. Finke, *J. Am. Chem. Soc.* **112**, 2419 (1990); S. A. Harder, W.-P. Lu, B. F. Feinberg, S. W. Ragsdale, *Biochemistry* **28**, 9080 (1989)].
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T Cell Awareness of Paternal Alloantigens During Pregnancy

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During pregnancy a semiallogeneic fetus survives despite the presence of maternal T cells specific for paternally inherited histocompatibility antigens. A mouse transgenic for a T cell receptor recognizing the major histocompatibility (MHC) antigen H-2K^b was used to follow the fate of T cells reactive to paternal alloantigens. In contrast to syngeneic and third-party allogeneic pregnancies, mice bearing a K^b-positive conceptus had reduced numbers of K^b-reactive T cells and accepted K^b-positive tumor grafts. T cell phenotype and responsiveness were restored after delivery. Thus, during pregnancy maternal T cells acquire a transient state of tolerance specific for paternal alloantigens.

In outbred species, inheritance of paternal histocompatibility antigens by the embryo results in genetic mismatches to the mother. The semiallogeneic fetus is in direct physical contact with uterine and blood-borne cells of the mother, and fetal rejection by the maternal immune system is prevented by mechanisms as yet undefined (*1*). In mice, midgestational placenta expresses paternal MHC antigens of the K and D loci (*1*, *2*); when grafted into maternal-strain recipients, it is rejected and induces sensitization to paternal alloantigens (*3*). However, neither ignorance nor tolerance of maternal T cells to paternal alloantigens has been conclusively shown. Impairment of T cell responses has been observed, but its selectivity to paternal alloantigens remains controversial (*1*, *4*, *5*). Midpregnant CBA mice, which are inbred, have unaltered expression of T cell receptor (TCR), CD4, and CD8 (*6*). However, phenotypic changes may go undetected because T cells specific for paternal alloantigens have low frequency in a normal T cell repertoire. Here, we used a TCR transgenic mouse model (Des-TCR) harboring a T cell repertoire skewed toward the paternal alloantigen H-2K^b (*7*) to take advantage of the high frequency of allospecific cytotoxic T cells as well as the ease of monitoring the transgenic TCR with clonotype-specific antibodies.

Virgin H-2^k Des-TCR transgenic females were mated with H-2^b C57BL/6 males, and K^b-specific T cells were phenotypically characterized during pregnancy. Nonspecific gestational effects (*8*) were controlled for by syngeneic and third-party allogeneic matings with H-2^k CBA or H-2^s ASW males (*9*), respectively. Midpregnant Des-TCR mice bearing a K^b-positive conceptus had reduced numbers of T cells with high expression of the clonotype (Fig. 1B, left) and six to nine times more clonotype-positive cells devoid of CD4 and CD8 (Fig. 1B, right) when compared to the results obtained for H-2^k syngeneic (Fig. 1C) and H-2^s third-party allogeneic (Fig. 1D) pregnancies. Therefore, maternal T cells specifically recognize paternal alloantigens.

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To determine whether T cell phenotypic changes in response to paternal K^b persist after delivery, we mated thymectomized H-2^k Des-TCR females with H-2^b C57BL/6 males. During midpregnancy, clonotype-positive T cells underwent phenotypic alterations (Fig. 2B) similar to those of nonthymectomized mice (Fig. 1B). After delivery, the expression of the clonotype, CD4, and CD8 in K^b-specific T cells (Fig. 2C) did not differ from that of control mice (Fig. 2A). Therefore, the alterations of mature peripheral T cells occur extrathymically and are reversed after delivery in the absence of a new thymic T cell input (*10*).

To dissect the role of B cells and $CD4^+$ T cells during pregnancy, we used Des-TCR SCID (severe combined immunodeficiency disease) mice. SCID mice are unable to autonomously rearrange immunoglobulin (Ig) and TCR genes but normally express a rearranged transgenic TCR (*11*). The peripheral lymphoid organs of H-$2^{d\times k}$ Des-TCR SCID mice were devoid of B cells, T cells expressing TCRs other than the transgenic clonotype, and $CD4^+Des^+$ T cells (Fig. 3B); this observation supports the hypothesis that the expression of a second TCR is required for positive selection of $CD4^+Des^+$ T cells (*12*). When mated with C57BL/6 males, H-$2^{d\times k}$ Des-TCR SCID mice gave birth to healthy litters of comparable size to those from syngeneic matings. Clonotype expression was reduced in H-2^b allogeneic pregnancies (Fig. 3D) in comparison with syngeneic pregnancies (Fig. 3E). Therefore, the encounter with paternal K^b is per se sufficient to perturb the phenotype of $CD8^+Des^+$ T cells, which rules out the hypotheses that fetal rejection is prevented by alloantibodies masking paternal MHC class I antigens on fetal target cells (*13*) and that $CD4^+$ T cells are of critical importance for successful pregnancies in this model. Production of cytokines such as transforming growth factor–β2 (*14*), interleukin (IL)-4, and IL-10 at the fetomaternal interface may induce T_H2-type $CD4^+$ T cells, thereby improving fetal survival (*15*). In Des-TCR SCID mice, $CD8^+$ T cells may have a similar function, as $CD8^+$ T cells reportedly produce heterogeneous patterns of cytokines (*16*). To assess whether placental sequestration accounted for the reduction of T cells expressing high clonotype levels, we analyzed fetoplacental tissues from midpregnant Des-TCR SCID mice by immunohistology. $CD8^+Des^+$ T cells were absent from placental and embryonic tissues during H-2^b allogeneic and syngeneic pregnancies (*17*); thus, clonotype-positive T cells were not trapped in the placenta. The site where T cells encounter paternal K^b remains undefined. Fetal cells leaking into the maternal circulation may provide an alternative source of paternal alloantigens (*18*).

To investigate whether tolerance to paternal alloantigens is induced during pregnancy and then reversed after delivery because of antigen elimination (*19*), we overlapped the putative window of tolerance with the immune challenge and used a phenomenon no longer reversible after the decline of tolerance as a readout system. The growth of P815-K^b tumor grafts was the criterion for K^b-specific T cell tolerance, because K^b-transfected H-2^d P815 mastocytoma cells are rejected by K^b-specific T cells in H-$2^{d\times k}$ Des-TCR mice and are accepted by K^b-tolerant mice (*20*). P815-K^b cells administered at the time of fetal implantation (*8*) generated a K^b-positive tumor mass in Des-TCR mice bearing a K^b-positive conceptus, but they were usually rejected during syngeneic and third-party allogeneic pregnancies (Fig. 4). P815-K^b tumor growth was also

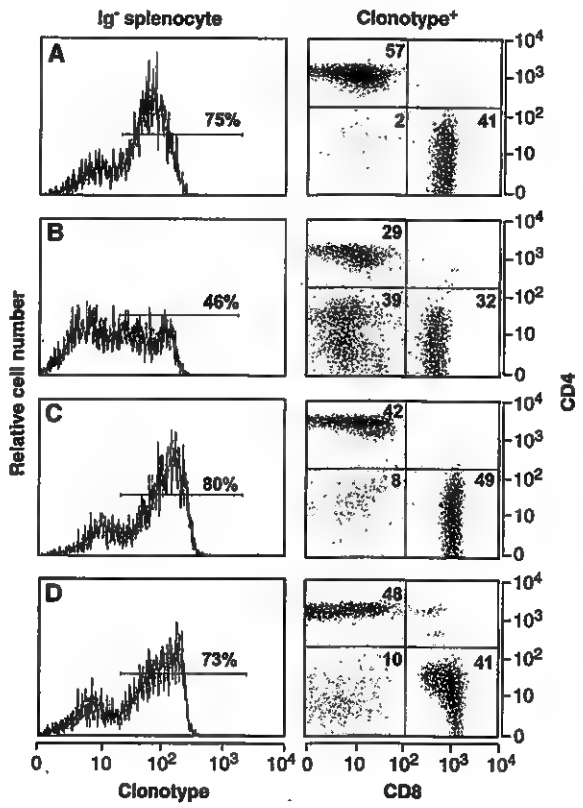


Fig. 1. Midgestational changes of K^b-specific T cell phenotype in response to paternal K^b. Three-color cytofluorimetric analyses (*9*) of B cell-depleted splenocytes from nonpregnant mice (**A**) and from midpregnant (days 9 to 11) H-2^k Des-TCR mice during H-2^b allogeneic pregnancies (**B**), H-2^k syngeneic pregnancies (**C**), and third-party H-2^s allogeneic pregnancies (**D**) are shown. Histograms represent clonotype expression; dot plots represent CD4 versus CD8 expression on gated clonotype-positive T cells. The data are representative of four experiments.

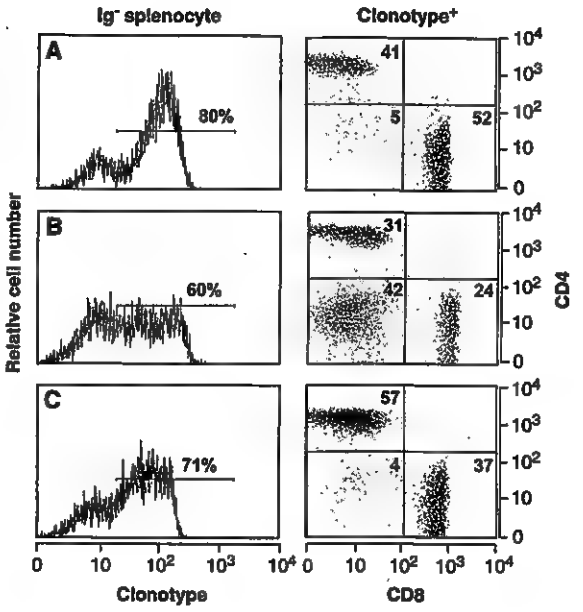


Fig. 2. Extrathymic occurrence and postpartum reversibility of T cell phenotypic changes in response to paternal K^b. Three-color cytofluorimetric analyses of B cell-depleted splenocytes (*9*) from adult thymectomized H-2^k Des-TCR mice under control conditions (**A**), on day 10 of H-2^b allogeneic pregnancies (**B**), and 3 days after delivery (**C**) are shown. Histograms represent clonotype expression; dot plots represent CD4 versus CD8 expression on gated clonotype-positive T cells. Thymectomy was performed at 4 weeks of age. The data are representative of three experiments.

observed in four of five Des-TCR mice injected on days 10 to 11 of H-2^b allogeneic pregnancies. After delivery (21 to 28 days), the ability to reject P815-K^b grafts was restored (four of four allogeneic pregnancies and three of three syngeneic pregnancies). Because K^b-positive tumor grafts were only accepted in the presence of a K^b-positive fetus, we conclude that pregnancy induces a transient state of T cell tolerance specific for the paternal alloantigens.

During pregnancy, paternal grafts may survive only if they bear MHC-peptide complexes identical to those of the fetus. Mismatches resulting from graft expression of tissue-specific peptides may recruit T cells reactive to these "nonfetal" components and may lead to graft rejection. Unlike mice harboring a normal T cell repertoire, Des-TCR transgenic mice may be unable to mount such responses because their repertoire is skewed toward the "tolerant" allospecificity (*21*). Recognition of maternal MHC-peptide complexes expressed by the fetus may theoretically block harmful T cell reactions against maternal autoantigens (*22*). Such extended T cell "awareness" to fetal components could in part explain why certain autoimmune diseases, such as multiple sclerosis and rheumatoid arthritis, undergo remission during pregnancy (*23*), apparently in the absence of general immunosuppression. If this hypothesis were true, understanding the unique features of T cell interactions with fetal cells would provide a powerful tool to reinstruct the immune system in the course of autoimmune diseases and transplant rejection.

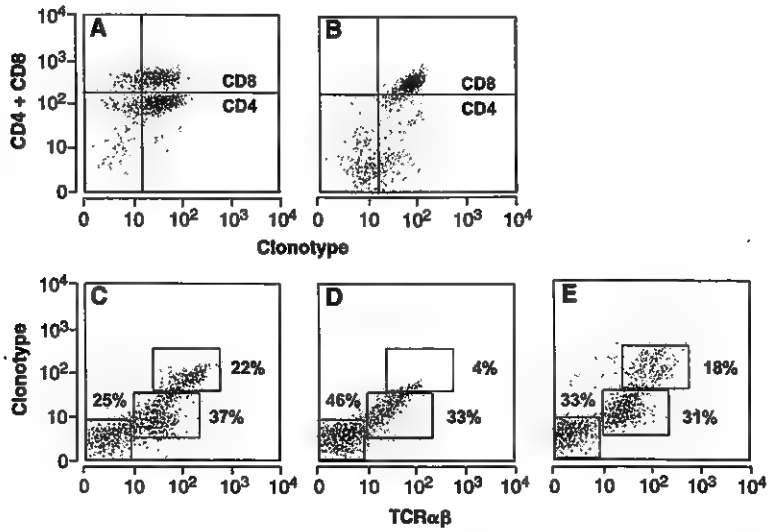
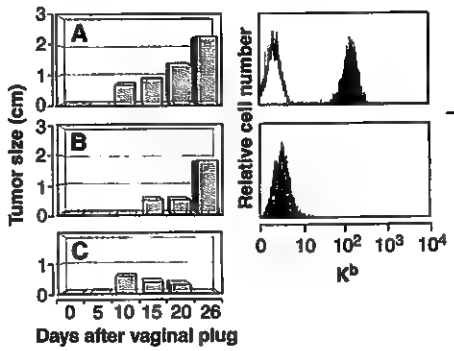


Fig. 3. Phenotypic alterations of CD8$^+$Des$^+$ T cells in response to paternal K^b in Des-TCR SCID mice. (**A** and **B**) Peripheral lymphoid organs of Des-TCR SCID mice are devoid of CD4$^+$Des$^+$ T cells. Dot plots represent clonotype versus CD4 and CD8 expression on B cell–depleted lymph nodes from H-2$^{d\times k}$ Des-TCR mice (A) and total lymph node cells from H-2$^{d\times k}$ Des-TCR SCID mice (B). (**C** through **E**) Dot plots represent TCRαβ versus clonotype expression on splenocytes from nonpregnant mice (C) and from midpregnant H-2$^{d\times k}$ Des-TCR SCID mice bearing H-2^b allogeneic (D) or syngeneic (E) concepti. The data are representative of three experiments.



Type of pregnancy	H-2^b all.	H-2^k syn.	H-2^s all.
A	15/19	2/14	2/9
B	3/19	5/14	1/9
C	1/19	7/14	6/9

Fig. 4. Impaired rejection of K^b-positive tumor grafts by Des-TCR mice bearing a K^b-positive conceptus. H-2$^{d\times k}$ Des-TCR mice were challenged with 1 × 10^5 P815-K^b tumor cells on days 3 to 5 of H-2^b allogeneic, H-2^k syngeneic, or third-party H-2^s allogeneic pregnancies. Representative examples of growth kinetics (left) and K^b expression of P815-K^b cells ex vivo (right) are shown. Three types of effects were observed: growth of a K^b-positive tumor (**A**), growth of a K^b-negative loss variant (**B**), and rejection (**C**); the table shows the incidence of these effects for the three pregnancy types. Because ≥95% of injected P815-K^b cells expressed K^b, the growth of the K^b-negative variants resulted from rejection of K^b-positive cells and in vivo immune selection. K^b-specific T cell responsiveness was significantly impaired ($P \leq 0.05$, Fisher exact test) during H-2^b allogeneic pregnancies when compared to syngeneic ($P = 0.004$) and third-party allogeneic ($P = 0.04$) pregnancies.

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6. All animals were kept under specific pathogen–free conditions. Allogeneic and syngeneic pregnancies were obtained by mating virgin H-2^k CBA females with H-2^b C57BL/6 and CBA males, respectively. A doubling of the number of total (*5*) and B cell–depleted splenocytes was observed during both allogeneic and syngeneic midpregnancies. Three-color cyto- fluorimetric analysis (*9*) revealed unchanged expression of TCRαβ, CD4, and CD8.
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9. Mating of Des-TCR mice with C57BL/6 males resulted in healthy litters of normal size. H-2^s ASW mice were selected as third-party allogeneic partners because T cells from Des-TCR mice do not respond to ASW stimulators in mixed leukocyte reactions. An increase in the number of total (60 × 10^6 versus 120 × 10^6 mean value) and B cell–depleted (35 × 10^6 versus 59 × 10^6 mean value) splenocytes was observed in midpregnant Des-TCR mice, irrespective of the male partner. Splenocyte counts reverted to normal after delivery. Splenocytes depleted of IgG-positive cells (sheep antibody to mouse IgG coupled to magnetic beads) were stained with the clonotype-specific Désiré-1 (*7*) monoclonal antibody (mAb) and with commercial (Gibco) mAbs specific for CD4 (H129.19), CD8 (53-6.7), and TCRαβ (H57-597). Biotinylated mAbs were revealed by streptavidin–Red 670 (Gibco). Analysis was performed on a FACScan cytometer (Becton Dickinson).
10. Pregnancy does not result in the permanent elimination of T cells specific for paternal alloantigens. However, the mechanisms responsible for the phenotypic alterations of K^b-specific T cells remain buried in the nonspecific fluctuations of spleen cell numbers typical of pregnancy (*9*). Reduction of Des-TCR T cells during midpregnancy may theoretically result from peripheral deletion, TCR down-regulation, or both. Restoration of the T cell phenotype after delivery may derive from up-regulation of TCR, CD4/CD8, or both, or from peripheral expansion of T cells maintaining high clonotype expression.
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mice carrying H-2^b allogeneic or H-2^k syngeneic pregnancies (day 10). Leukocytes were identified by a CD45-specific mAb (30F11.1, Pharmingen); T cells were identified by an unconjugated rat clonotype-specific mAb (B20.2.2) and by commercial (Gibco) biotinylated mAbs specific for CD3 (29B) and TCR$\alpha\beta$ (H57-597). Unconjugated primary antibodies were detected with a biotinylated sheep antibody to rat serum. Biotinylated antibodies were revealed by a streptavidin-biotinylated peroxidase complex. The numbers of CD45-positive cells were comparable in midpregnant allogeneic and syngeneic uteri. Maternal T cells were numerous in the decidua but were absent from the placenta and fetus.

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20. H-2^d P815-K^b mastocytoma cells implanted subcutaneously are rejected by H-2$^{d\times k}$ Des-TCR mice but are accepted by Des-TCR mice tolerant to K^b expressed under the Keratin-IV promoter (2.4Ker.K^b × Des-TCR) (*24*). P815-K^b elimination is mediated by Des-TCR T cells. In vivo depletion of Des-TCR T cells by Désiré-1 mAb, but not of natural killer cells by NK1.1 mAb, blocked tumor rejection (A. Limmer, G. J. Hämmerling, B. Arnold, unpublished data). Rejection was verified for more than 3 months. When the tumor diameter exceeded 2 cm after increasing for more than two consecutive measurements, tumor-bearing mice were killed, and K^b expression was tested on P815-K^b cells ex vivo.
21. Among all peptides extracted from K^b molecules [C57BL/6 spleen, EL-4, or RMA (H-2^b) tumor cells] and separated by high-performance liquid chromatography, only one and the same peptide fraction sensitized RMA-S cells for recognition by a Des-TCR–positive clone. Therefore, the Des-TCR–positive clone recognizes only one (or few) peptides in the context of K^b. The peptide sequence is currently being investigated (A. Guimezanes and A.-M. Schmitt-Verhulst, personal communication).
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Relaxation of Arterial Smooth Muscle by Calcium Sparks

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Local increases in intracellular calcium ion concentration ($[Ca^{2+}]_i$) resulting from activation of the ryanodine-sensitive calcium-release channel in the sarcoplasmic reticulum (SR) of smooth muscle cause arterial dilation. Ryanodine-sensitive, spontaneous local increases in $[Ca^{2+}]_i$ (Ca^{2+} sparks) from the SR were observed just under the surface membrane of single smooth muscle cells from myogenic cerebral arteries. Ryanodine and thapsigargin inhibited Ca^{2+} sparks and Ca^{2+}-dependent potassium (K_{Ca}) currents, suggesting that Ca^{2+} sparks activate K_{Ca} channels. Furthermore, K_{Ca} channels activated by Ca^{2+} sparks appeared to hyperpolarize and dilate pressurized myogenic arteries because ryanodine and thapsigargin depolarized and constricted these arteries to an extent similar to that produced by blockers of K_{Ca} channels. Ca^{2+} sparks indirectly cause vasodilation through activation of K_{Ca} channels, but have little direct effect on spatially averaged $[Ca^{2+}]_i$, which regulates contraction.

Myogenic arteries control blood flow in the brain and respond to changes in intravascular pressure. Increased intravascular pressure causes a graded membrane potential depolarization of smooth muscle cells and arterial constriction (myogenic tone) (*1–3*). Small, pressurized cerebral arteries dilate when the membrane potential of the smooth muscle cells is made more negative over the physiological range of membrane potentials (−60 to −30 mV), because steady Ca^{2+}-influx through dihydropyridine-sensitive, voltage-dependent Ca^{2+} channels declines (*2–4*). Ca^{2+} entry at physiological membrane potentials affects spatially averaged $[Ca^{2+}]_i$ in arterial smooth muscle (*3, 4*). Although ryanodine-sensitive Ca^{2+}-release channels directly contribute to the global $[Ca^{2+}]_i$ transient and contraction in cardiac muscle (*5*), their functional role in smooth muscle has not been established (*4, 6, 7*). We monitored elementary ryanodine-sensitive Ca^{2+}-release events (Ca^{2+} sparks) from smooth muscle SR by measuring rapid local changes in $[Ca^{2+}]_i$ in smooth muscle cells isolated from resistance-sized cerebral arteries. We provide evidence that ryanodine-sensitive Ca^{2+}-release channels in smooth muscle SR, unlike their counterparts in cardiac and skeletal muscle, have a central role in limiting muscle contraction by activating K_{Ca} channels.

Single smooth muscle cells were isolated enzymatically from myogenic cerebral (100- to 150-μm in diameter posterior and middle cerebral) arteries from rat (*8*). We used a laser scanning confocal microscope and the fluorescent Ca^{2+} indicator fluo-3 (*9*) to detect Ca^{2+} sparks in single cells bathed in physiological salt solution (Figs. 1 and 2). The mean rise-time and half-time of decay of Ca^{2+} sparks were 20.2 ± 2.3 ms and 48.0 ± 2.6 ms (n = 11), respectively (Fig. 1). The mean peak $[Ca^{2+}]_i$ during the Ca^{2+} spark was 303 ± 27 nM (assuming 100 nM resting Ca^{2+}) (Fig. 1) (*10*). The mean spread of the spark at the peak was 2.38 ± 0.14 μm (n = 11) (Fig. 1) (*10*), corresponding to 0.8% of the surface area of the cell membrane (*11*).

Ryanodine, which inhibits SR Ca^{2+}-release channels at micromolar concentrations (*5, 6*), blocked Ca^{2+} sparks in smooth muscle cells (Fig. 2B). Ca^{2+} sparks were not observed in cells exposed to 10 μM ryanodine and the Ca^{2+} channel agonist Bay K 8644, whereas 88% of cells treated with Bay K 8644 alone had Ca^{2+} sparks. Ca^{2+} sparks were not observed in cells exposed to thapsigargin (1 μM), which inhibits Ca^{2+} uptake into the SR by the Ca^{2+}-ATPase (*12*). Application of cadmium (200 μM), which immediately blocks voltage-dependent Ca^{2+} channels, did not immediately block Ca^{2+} sparks in our cells (n = 7), a finding similar to that observed in quiescent heart muscle cells (*5*). However, prolonged exposure to Bay K 8644 increased Ca^{2+}-spark occurrence (Fig. 2B). The majority of Ca^{2+} sparks (59%) arose close to the sarcolemmal surfaces (within 1 μm) of the smooth muscle cells (Fig. 2C). The Ca^{2+} sparks that were detected in the middle of the line-scan may still have arisen at the sarcolemmal surface because smooth muscle cells have infoldings of the surface membranes (caveolae). These results suggest that most Ca^{2+} sparks in smooth muscle cells from resistance-sized cerebral arteries result from the opening of ryanodine-sensitive Ca^{2+}-release channels in SR just under the cell membrane.

The proximity of the Ca^{2+} sparks to the cell surface raises the possibility that the Ca^{2+} spark serves as an intracellular signal to the sarcolemmal membrane. Ca^{2+}-activated K^+ (K_{Ca}) channels that exist in this membrane (*2, 3*) should be activated by the

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local increase in $[Ca^{2+}]_i$ produced by the Ca^{2+} sparks. To examine this possibility, we used the perforated-patch method to measure membrane currents in intact single cells (*13*). The membrane potential was held at −40 or −30 mV, similar to that of smooth muscle cells in the intact pressurized artery and in freshly dissociated smooth muscle cells (*1*–*3*). Outward current transients were observed (Figs. 1 to 3) with a time course similar to that of the Ca^{2+} spark and were completely inhibited by the application of ryanodine (10 μM) (n = 6) (Fig. 3A) or thapsigargin (100 nM) (n = 3) (Fig. 3B), suggesting that they were activated by the local $[Ca^{2+}]_i$ increase produced by individual Ca^{2+} sparks. Tetraethylammonium ions (TEA^+) (1 mM) (Fig. 3C) and

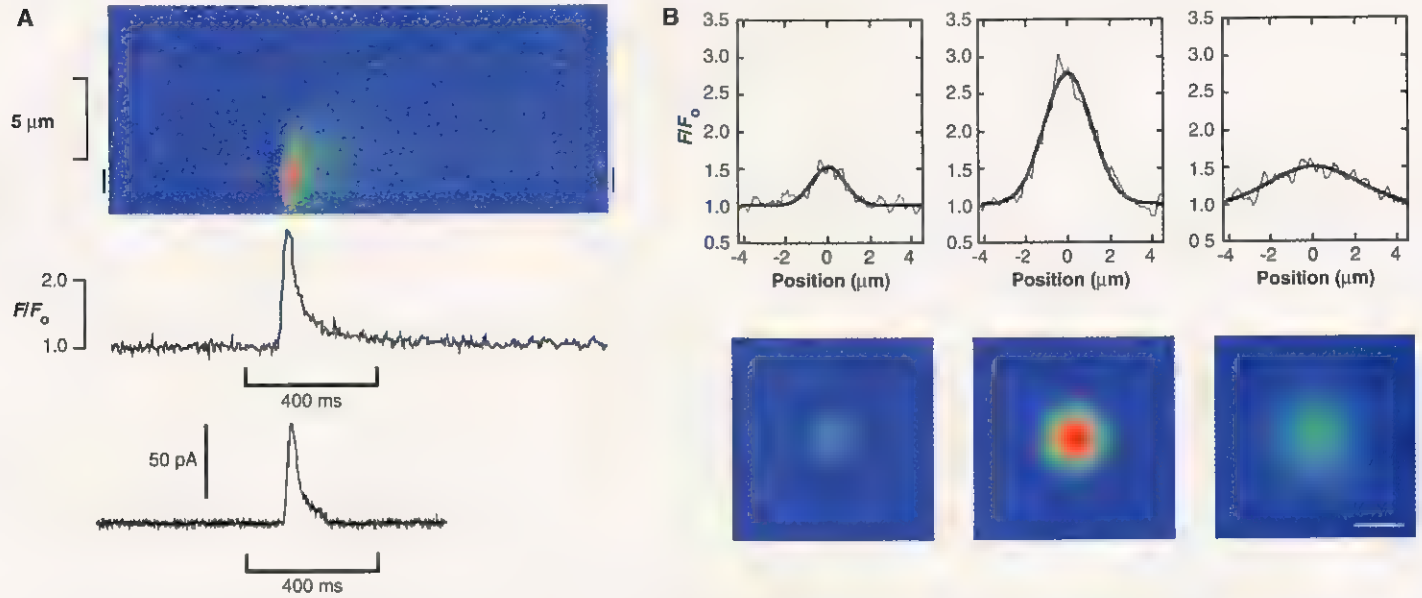
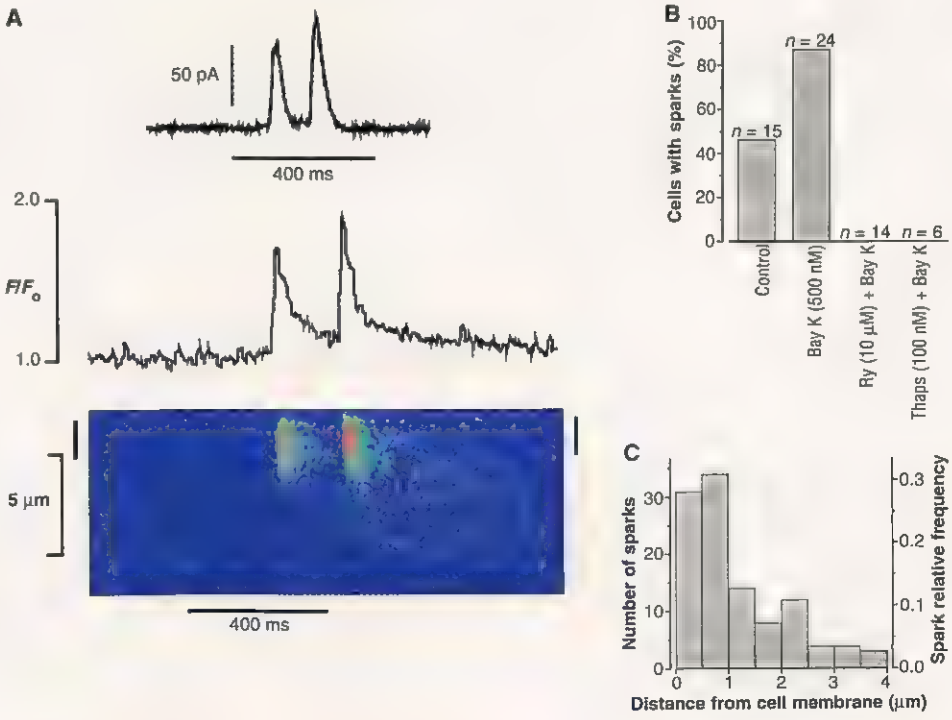


Fig. 1. Spatial-temporal characteristics of a Ca^{2+} spark in a smooth muscle cell from a rat posterior cerebral artery. (**A**) Confocal line-scan image of a fluo-3–loaded cerebral artery smooth muscle cell, with the time course indicated below. The fluorescence time course of the Ca^{2+}-spark was averaged over the region indicated by the bar. Bottom trace shows an example of a spontaneous transient outward current (STOC) from a different cell (at −40 mV) (Fig. 3). Each line-scan image is a plot of fluorescence along a scanned line (that is, position) on the ordinate versus time (on the abscissa) (*5*). The line-scan image duration was 1536 ms, and each line was 6 ms. (**B**) Spatial-temporal characteristics of the spark shown in (A). (**Upper panels**) Spatial distribution of Ca^{2+} fit by a Gaussian (solid line), relative to the initiation point of the spark. Shown are the spatial distributions at the first indication of an increase in Ca^{2+} (left), at the peak Ca^{2+} (middle), and 66 ms later (right). The full width at half-maximal $[Ca^{2+}]$ at the three time points of the spark life cycle were 1.84, 2.65, and 4.99 μm. (**Lower panels**) Corresponding estimated spread of a spark by rotation of the fit Gaussian 360°. Bar, 3 μm. Single smooth muscle cells were isolated enzymatically from 100-μm-diameter myogenic posterior cerebral arteries from rat as described (*8*). Bath solution: 6 mM KCl, 134 mM NaCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM Hepes, and 10 mM glucose (pH 7.4) at room temperature.

Fig. 2. Calcium sparks in single smooth muscle cells isolated from myogenic cerebral arteries: Inhibition of Ca^{2+} sparks by ryanodine and thapsigargin and localization next to the surface membrane. (**A**) Line-scan image illustrating two sparks at the edge, with the time course of the two sparks and an example of two STOCs (from a different cell, at −40 mV) above the image. Bar on line-scan image, 1.5 μm. (**B**) Percentage of cells exhibiting one or more sparks during 30-s scanning with Bay K 8644 (500 nM), thapsigargin (Thaps; 1 μM) + Bay K, and ryanodine (Ry; 10 μM) + Bay K. The cells were incubated with each drug for at least 10 min before being examined for sparks. Each cell was scanned for a average total time of 30 s. Longer scanning resulted in bleaching of the dye. Assuming that 1% of the cell volume was scanned for 30 s and a spark frequency of 1/s (on the basis of STOC measurements), a spark should have been observed in about 30% of the control cells. The total number of cells examined under each condition is indicated above each bar. (**C**) Frequency of sparks as a function of distance from edge of the cell. The edges of the cells correspond to the upper and lower edges of the line-scan image.



iberiotoxin (100 nM) (Fig. 3D) completely blocked the currents, confirming the identification of the current source as K_{Ca} channels (*2*, *3*, *14*). Ryanodine-sensitive, spontaneous transient outward currents (STOCs) through K_{Ca} channels have been observed in a number of other types of smooth muscle (*15*, *16*). At −40 mV, the STOCs we measured had a mean amplitude of 21.5 ± 6.4 pA (n = 7), a mean duration of 64.9 ± 7.9 ms, a mean rise time of 17.1 ± 1.6 ms, and a mean frequency of 1.28 ± 0.42 Hz (n = 6) in intact quiescent cells. Thus, the kinetics of the STOCs were similar to those of the Ca^{2+} sparks. Ryanodine appeared not to inhibit STOCs by blocking K_{Ca} channels, because neither ryanodine (10 μM) nor thapsigargin (1 μM) decreased the open-state probability or unitary current of single K_{Ca} channels in excised patches (Fig. 3 legend). The minimum number of K_{Ca} channels activated during a STOC can be estimated by dividing the mean STOC amplitude (21.5 pA) at −40 mV by the mean single-channel current (1.6 ± 0.1 pA; n = 3) (*17*). The results support the idea that a single ryanodine-sensitive Ca^{2+} spark from the SR activates at least 13 K_{Ca} channels to produce a single STOC.

It would thus be expected that Ca^{2+} sparks, by activating K_{Ca} channels, would hyperpolarize the membrane potential of myogenic cerebral arteries and so result in vasodilation (*2*, *3*, *16*, *18*). The myogenic response to an increase in intravascular

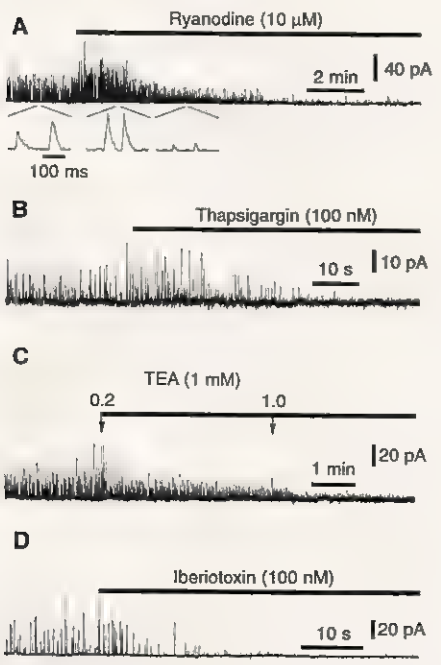


Fig. 3. Inhibition of spontaneous transient outward currents by ryanodine, thapsigargin, TEA^+, and iberiotoxin. (**A**) Ryanodine (10 μM) block of STOCs. (Below: expanded time course before, shortly after, and during addition of ryanodine before cessation of STOCs.) Ryanodine has no direct effect on K_{Ca} channels [NP_o (ryanodine)/NP_o (control) = 0.97 ± 0.02 (SE; n = 3)], and unitary currents at 0 mV were 5.94 ± 0.3 pA (control) and 5.71 ± 0.34 pA (ryanodine). (**B**) Thapsigargin (100 nM) block of STOCS. Thapsigargin has no direct effect on K_{Ca} channels [NP_o (thapsigargin)/NP_o (control) = 0.928 ± 0.18 (SE; n = 4)], and unitary currents at 0 mV were 6.1 ± 0.2 pA (control) and 6.1 ± 0.3 pA (thapsigargin). (**C**) Tetraethylammonium (TEA^+) block of STOCs. TEA^+ at 0.2 mM reduced STOC amplitudes by 50%, which is the same as the concentration of TEA^+ required to inhibit single K_{Ca} channels by 50% (*14*). (**D**) Iberiotoxin (100 nM) block of STOCs. Whole-cell currents in single smooth muscle cells isolated from myogenic cerebral arteries of rat were measured with the perforated-patch configuration of the whole-cell recording technique. Bath solution: 6 mM KCl, 134 mM NaCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM Hepes, 10 mM glucose (pH 7.4); pipette solution: 30 mM KCl, 110 mM potassium aspartate, 10 mM NaCl, 1 mM $MgCl_2$, 50 μM EGTA, amphotericin (200 mg/ml) (pH 7.2) at room temperature.

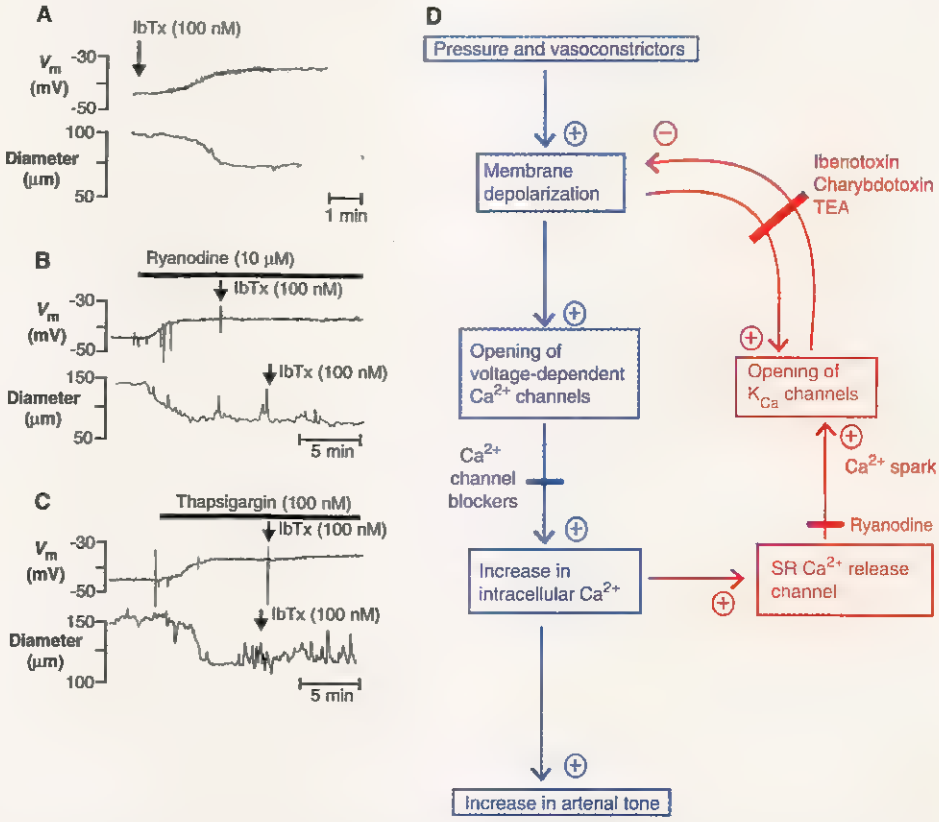


Fig. 4. Depolarization and constriction of rat myogenic cerebral arteries with tone by inhibitors of K_{Ca} channels (iberiotoxin) (**A**) and Ca^{2+} sparks [ryanodine (**B**) and thapsigargin (**C**)]. Cerebral arterial pressure was 60 mm Hg in all experiments. Iberiotoxin alone depolarizes and constricts. Ryanodine (10 μM) depolarizes and constricts, and addition of iberiotoxin (arrow) had no effect on membrane potential and diameter in the presence of ryanodine (n = 5). Thapsigargin (100 nM) depolarizes and constricts, but iberiotoxin (100 nM) was without effect in the presence of thapsigargin. The mean diameter of the arteries at 60 mm Hg dilated with 100 nM nisoldipine was 228 ± 43 μm (n = 17). The diameter of the pressurized arteries was measured by a video image analyzer (Living Systems Instruments, Burlington, Vermont), and the membrane potential was measured with conventional microelectrodes as described (*2*). Bath solution: 119 mM NaCl, 4.7 mM KCl, 24 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.6 mM $CaCl_2$, 1.2 mM $MgSO_4$, 0.023 mM EDTA, 11 mM glucose (pH 7.4) with continuous bubbling with 95% O_2–5% CO_2 at 37°C. The diameter and membrane potential measurements were made in different arteries. (**D**) Proposed model for the roles of K_{Ca} channels and ryanodine-sensitive Ca^{2+}-release channels in the SR (Ca^{2+} sparks) in the regulation of arterial tone. In this model, Ca^{2+} sparks have a key role in the negative-feedback regulation of arterial tone through activation of K_{Ca} channels (right limb of diagram in red). Intravascular pressure causes a maintained membrane potential depolarization and constriction (myogenic tone) of small myogenic cerebral arteries (*1*–*3*). Myogenic tone is blocked by the removal of external Ca^{2+}, voltage-dependent Ca^{2+} channel blockers, or membrane hyperpolarization (*1*–*3*). Activation of Ca^{2+} channels increases Ca^{2+} entry and spatially averaged Ca^{2+} in the smooth muscle (*3*, *22*, *29*). This small increase in spatially averaged Ca^{2+} produces a steep increase of force (*22*) (left limb of diagram in blue), which would result in an increase of SR Ca^{2+} content. This should increase Ca^{2+} spark frequency and amplitude and thereby activate K_{Ca} channels (*23*). Other important elements in the control of arterial tone not shown include inositol trisphosphate (IP_3)–induced Ca^{2+} release, Ca^{2+} extrusion and uptake mechanisms, and mechanisms to change Ca^{2+} sensitivity (*7*). This model also suggests a mechanism by which vasodilators and vasoconstrictors could regulate arterial tone through modulation of Ca^{2+} spark frequency and amplitude.

pressure from 10 mm Hg to 60 to 80 mm Hg involves the depolarization of the smooth muscle membrane potential from about −60 to about −40 mV, the opening of voltage-dependent Ca^{2+} channels, increased $[Ca^{2+}]_i$, and the consequent constriction of the vessel by about 30 to 40% (*1–3, 7, 19*). Blockers of K_{Ca} channels (such as charybdotoxin and iberiotoxin) depolarize the membrane potential of smooth muscle cells by 7 to 9 mV and constrict myogenic pressurized cerebral arteries by about 40% (*2*) (Fig. 4A). The mean membrane potential at a pressure of 60 mm Hg was −43.9 ± 1.4 mV (n = 12). Iberiotoxin caused the membrane potential to depolarize by 8.6 ± 2.3 mV (n = 3) at 60 mm Hg and 9 ± 2 mV at 80 mm Hg (n = 6) and decreased arterial diameter by 41 ± 9% (n = 6) (*2*).

If Ca^{2+} sparks are major activators of K_{Ca} channels in pressurized cerebral arteries with tone, then inhibitors of Ca^{2+} release from the SR (ryanodine and thapsigargin) and blockers of K_{Ca} channels should depolarize and constrict pressurized cerebral arteries to a similar extent. In the presence of ryanodine and thapsigargin, iberiotoxin should have little effect on membrane potential and diameter, because the localized increases in $[Ca^{2+}]_i$ (that is, Ca^{2+} sparks) that activate K_{Ca} channels would be eliminated. Ryanodine (10 μM), which blocks the SR Ca^{2+}-release channel, depolarized pressurized (60 mm Hg) posterior cerebral arteries from −43.9 mV to −36.6 ± 0.8 mV (n = 6), or by 7.3 mV, and constricted these arteries from 121 ± 12 μm to 81 ± 7 μm (n = 6), or by 33% (Fig. 4B). In contrast to the depolarization and constriction of cerebral arteries caused by iberiotoxin alone (*2*), this K_{Ca} channel blocker had no effect on membrane potential or diameter after application of ryanodine (n = 5) (Fig. 4B) (*20*). Similarly, after thapsigargin had been applied [to block SR Ca-ATPase (*12*)], the addition of iberiotoxin did not affect membrane potential or diameter (Fig. 4C). Thapsigargin (100 nM) alone depolarized and constricted the pressurized (60 mm Hg) cerebral arteries by 6.9 ± 1.4 mV (n = 4) and from 122 ± 13 mV (n = 5) to 96 ± 12 mV (n = 4), respectively (n = 3) (Fig. 4C). Furthermore, the effects of thapsigargin were tested in arteries denuded of the endothelium to eliminate any possible complications from alterations in nitric oxide release (*21*). Thapsigargin also constricted these pressurized cerebral arteries from 95 ± 14 μm to 62 ± 10 μm (n = 3), or by 35%. Another inhibitor of the SR Ca-ATPase, cyclopiazonic acid (10 μM), also constricted pressurized cerebral arteries (from 123 ± 10 μm to 77 ± 3 μm) (n = 3). These results suggest that blockers of Ca^{2+} sparks depolarize and constrict myogenic cerebral arteries by inhibiting K_{Ca} channels.

An increase in global $[Ca^{2+}]_i$ caused by raised intravascular pressure is due to Ca^{2+} influx through voltage-sensitive Ca^{2+} channels and results in an increase in force generation (*7, 22*) by means of myosin light chain kinase activated by Ca^{2+}-calmodulin (Fig. 4D). This modest increase in $[Ca^{2+}]_i$ (compared with the large local increase in $[Ca^{2+}]$ produced by a Ca^{2+} spark) has little direct effect on K_{Ca} channels (*17*) but should produce an increase in SR Ca^{2+} content and in Ca^{2+}-spark amplitude and frequency (*23*). Ca^{2+} sparks increase local $[Ca^{2+}]$ sufficiently to activate K_{Ca} channels, which hyperpolarize the cell. Ca^{2+} sparks could also affect other types of Ca^{2+}-sensitive processes (including ion channels) (*24*). Because the Ca^{2+} sparks are highly localized and occur at a low rate (about 1 per second per cell as inferred from the STOC frequency), they have little effect on spatially averaged Ca^{2+} within a cell (*4, 25*) and therefore do not cause contraction. Ryanodine-sensitive Ca^{2+} sparks occurred primarily next to the surface membrane, consistent with previous studies indicating that much of the SR in smooth muscle is adjacent to the sarcolemmal membrane (*7, 26, 27*). Therefore, localized large increases in $[Ca^{2+}]$ produced by Ca^{2+} sparks can increase K_{Ca} channel activity (*17*) and thereby hyperpolarize and relax small myogenic cerebral arteries (*2, 3*) (Fig. 4D). Regulation of the Ca^{2+} spark frequency is another means to control arterial diameter and presumably will depend on factors that regulate the opening rate of the ryanodine receptors (SR Ca^{2+}-release channels), such as the phosphorylation state and $[Mg^{2+}]$.

Blocking Ca^{2+} sparks or K_{Ca} channels in pressurized, myogenic arteries would then cause membrane depolarization and vasoconstriction (*16, 27*) (Fig. 4). The lack of effect of iberiotoxin in the presence of ryanodine or thapsigargin (that is, when Ca^{2+} sparks are blocked) (Fig. 4, B and C) suggests that average cytoplasmic Ca^{2+} in the absence of Ca^{2+} sparks does not cause sufficient activation of K_{Ca} channels to regulate smooth muscle membrane potential (Fig. 4D). However, inhibitors of voltage-dependent Ca^{2+} channels decrease average $[Ca^{2+}]_i$ and relax myogenic cerebral and skeletal muscle arteries in the presence of ryanodine (*28*). These findings indicate that Ca^{2+} entry can influence spatially averaged $[Ca^{2+}]_i$, which in turn regulates contraction (Fig. 4D). In conclusion, we propose that the ryanodine-sensitive Ca^{2+}-release channel has a key function in controlling the diameter of small myogenic arteries through the regulation of K_{Ca} channels. These results suggest a mechanism for control of vasodilation and constriction through modulation of the amplitude and frequency of Ca^{2+} sparks.

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9. A Bio-Rad MRC600 (Cambridge, MA) confocal scanning head connected to a Nikon Diaphot microscope was used to image the cells with COMOS and SOM software (Bio-Rad). Images were acquired in line-scan mode of the confocal microscope; this mode repeatedly scans a single line through the cell every 6 ms. The line-scan traverses the entire width of a cell. The line-scan results are displayed vertically and each line is added to the right of the preceding line to form the line-scan image. In these images, time is in the horizontal direction (left to right) and position along the scan line is given by the vertical displacement. The resolution of the microscope is approximately 0.4 μm by 0.4 μm (x and y) by 0.8 (z) μm with a Zeiss Neofluor 63 1.25 NA objective, as measured with 0.09-μm fluorescent beads (Molecular Probes, Eugene, OR). IDL software (Boulder, CO) was used for data analysis. The fluorescence record was normalized by dividing the fluorescence traces by the average fluorescence during the prestimulus period. Calibration of the fluo-3 signal was done as described (*5*). The cells were loaded with fluo-3 by a 10-min incubation with 5 μM fluo-3 AM (Molecular Probes) followed by a 30-min wash. The site of origin of a Ca^{2+} spark was determined as the center of the spark at the time of its initiation.
10. Although the peak $[Ca^{2+}]_i$ of a spark and the spatial profile of the Ca^{2+} sparks are similar in cerebral artery smooth muscle cells and cardiac myocytes (*5*), the kinetics of Ca^{2+} sparks in smooth muscle cells are slower. The slower kinetics could arise from different ryanodine-sensitive Ca^{2+}-release channel kinetics or altered properties of the SR Ca-ATPases and Ca^{2+} buffers. The increase of $[Ca^{2+}]_i$ occurring during a Ca^{2+} spark is consistent with either the opening of one or the coordinated opening of a small number of colocalized ryanodine-sensitive Ca^{2+}-release channels (*5*).
11. Single smooth muscle cells from posterior cerebral

arteries of rat have a mean membrane capacitance of 10.8 ± 0.4 pF (n = 47) (*8*), corresponding to a membrane surface area of about 1080 μm². The fraction of the surface area experiencing a spark was estimated as the surface area of a hemisphere (about 9 μm²) with a radius (1.19 μm) of the mean spread of a spark divided by the cell surface area (1080 μm²). The volume of a single cell was estimated to be about 1 pl.

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18. Very few K^+ channels need to open to cause a membrane hyperpolarization of arterial smooth muscle (*3*), because arterial smooth muscle cells have high input resistances (10 gigohms) at physiological membrane potentials in the absence of STOCs. Therefore, the membrane potential would hyperpolarize towards the K^+ equilibrium potential (−85 mV) during an average STOC (*16*). STOCs in smooth muscle cells in the arterial wall would sum to cause a graded membrane potential hyperpolarization. The average membrane potential would reflect the contribution of K_{Ca} conductance caused by Ca^{2+} sparks and other conductances.
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25. A single Ca^{2+} spark at its peak occupies about 7 fl with an average Ca^{2+} of about 300 nM, and occurs in 0.7% of the volume of a smooth muscle cell. A Ca^{2+} spark would thus have an insignificant effect on spatially averaged $[Ca^{2+}]_i$. The highest STOC rate that we observed was 9/s, which would also have little effect on $[Ca^{2+}]_i$, assuming that this corresponded to 9 sparks/s. Even if the increase of $[Ca^{2+}]_i$ lasted 100 ms, the average Ca^{2+} in the cell would still change by <2 nM.
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28. Our results are consistent with the ryanodine- and thapsigargin-induced constrictions of the artery being caused by membrane depolarization, which increases Ca^{2+} entry through voltage-dependent Ca^{2+} channels (*3*) (Fig. 4D), particularly because the iberiotoxin-, ryanodine-, and thapsigargin-induced constrictions were similar and not additive. In support of this mechanism, we observed that ryanodine and thapsigargin had no effect on arterial diameter in the presence of the Ca^{2+} channel blocker, nisoldipine (n = 6). Further, nifedipine has been shown to block ryanodine- and cyclopiazonic acid–induced increases in spatially averaged $[Ca^{2+}]_i$ and constrictions of myogenic skeletal muscle arterioles [J. Watanabe *et al.*, *Circ. Res.* **73**, 465 (1993)].
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Localization of Protein Implicated in Establishment of Cell Type to Sites of Asymmetric Division

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Asymmetric division in *Bacillus subtilis* generates progeny cells with dissimilar fates. SpoIIE, a membrane protein required for the establishment of cell type, was shown to localize near sites of potential polar division. SpoIIE initially localizes in a bipolar pattern, coalescing at marks in the cell envelope at which asymmetric division can take place. Then, during division, SpoIIE becomes restricted to the polar septum and is lost from the distal pole. Thus, when division is complete, SpoIIE sits at the boundary between the progeny from which it dictates cell fate by the activation of a cell-specific transcription factor.

A fundamental challenge in developmental biology is to understand how cells of one type differentiate into other, more specialized types of cells (*1*). One way specialization occurs is by asymmetric cell division in which a progenitor cell gives rise to two dissimilar progeny that follow different pathways of differentiation. A simple system in which the relation between cell fate and asymmetric division has been investigated is spore formation in *Bacillus subtilis* (*2*). Spore formation involves an asymmetric cell division in which a septum is formed near one pole of the developing cell (the "sporangium"), partitioning it into unequal-sized progeny called the forespore (the small cell) and the mother cell. Crucial to the establishment of the dissimilar fates of the progeny is a putative integral membrane protein (*3*) called SpoIIE (*4*), whose synthesis commences shortly before asymmetric division (*5*).

SpoIIE is not needed for the formation of the polar septum (*6*, *7*) but is required for the activation in the forespore of a transcription factor called σ^F (*4*). The σ^F factor is present in the predivisional sporangium but is held in an inactive complex prior to septation by the inhibitory protein SpoIIAB (*8*). After the polar septum is formed, σ^F continues to be held in an inactive complex in the mother cell, while SpoIIE triggers the release of σ^F from SpoIIAB in the forespore. The mechanism by which SpoIIE activates σ^F has

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been unknown until now (*9*) but is inferred to involve an additional protein (SpoIIAA) to which SpoIIAB binds in order to release σ^F (*4*, *8*). Freed from inhibition in the forespore as a result of the action of SpoIIE, σ^F sets in motion a chain of events that determine the subsequent fate of the two progeny cells (*2*).

Because of its role in the establishment of cell type, we investigated the subcellular localization of SpoIIE. Polyclonal antibodies were raised against the COOH-terminal region of SpoIIE (*10*) and used to examine the spatial distribution of the protein by immunofluorescence microscopy (*11*). Cells were collected at various times early after the onset of sporulation, fixed, and permeabilized for labeling with antibodies. In some sporangia (designated class i; Fig. 1A) antibody labeling was bipolar, with zones of fluorescence (red color) located near both ends of the cells (*12*). Sporangia with the bipolar pattern of SpoIIE immunostaining were at a very early stage of development because (as revealed by DAPI staining) (*13*) they lacked a condensed forespore chromosome. As sporulation progresses and a polar septum is formed, one chromosome becomes tightly packed into the forespore and is recognized as a region of intense DAPI staining near one pole of the sporangium (*14*). An additional indication that class i sporangia were at a very early stage was that σ^F-directed gene expression had not yet commenced. This was determined by also staining the sporangia, which contained *lacZ* fused to a gene under the control of σ^F, with antibodies to β-galactosidase (β-Gal) (Fig. 1B, representing the same field of cells as in Fig. 1A). The absence of β-Gal staining from class i sporangia indicated that little or no σ^F-directed gene expression had occurred.

Class ii sporangia contained immunostaining of SpoIIE preferentially at one pole (Fig. 1, A, C, and D). Class ii sporangia are more advanced than class i cells because a condensed forespore chromosome was evident (*13*). Also, a low level of β-Gal from σ^F-directed synthesis of the enzyme could be detected (seen as white-to-pink color in Fig. 1B and, as a result of the absence of DAPI fluorescence in this photograph, the yellow color in Fig. 1D). The bipolar pattern (class i) of SpoIIE staining was observed with high frequency among sporangia that had not yet formed a condensed forespore chromosome, and the forespore-specific pattern (class ii) was frequent among sporangia that contained a condensed chromosome (Fig. 2). Finally, class iii sporangia represented a third, more advanced stage of development. In these sporangia, little or no SpoIIE could be detected at either pole. Rather, class iii sporangia exhibited a fully condensed forespore chromosome (evident from photographs of DAPI staining alone) and substantial accumulation of β-Gal (green-blue color; Fig. 1B).

Thus, SpoIIE localizes near both ends

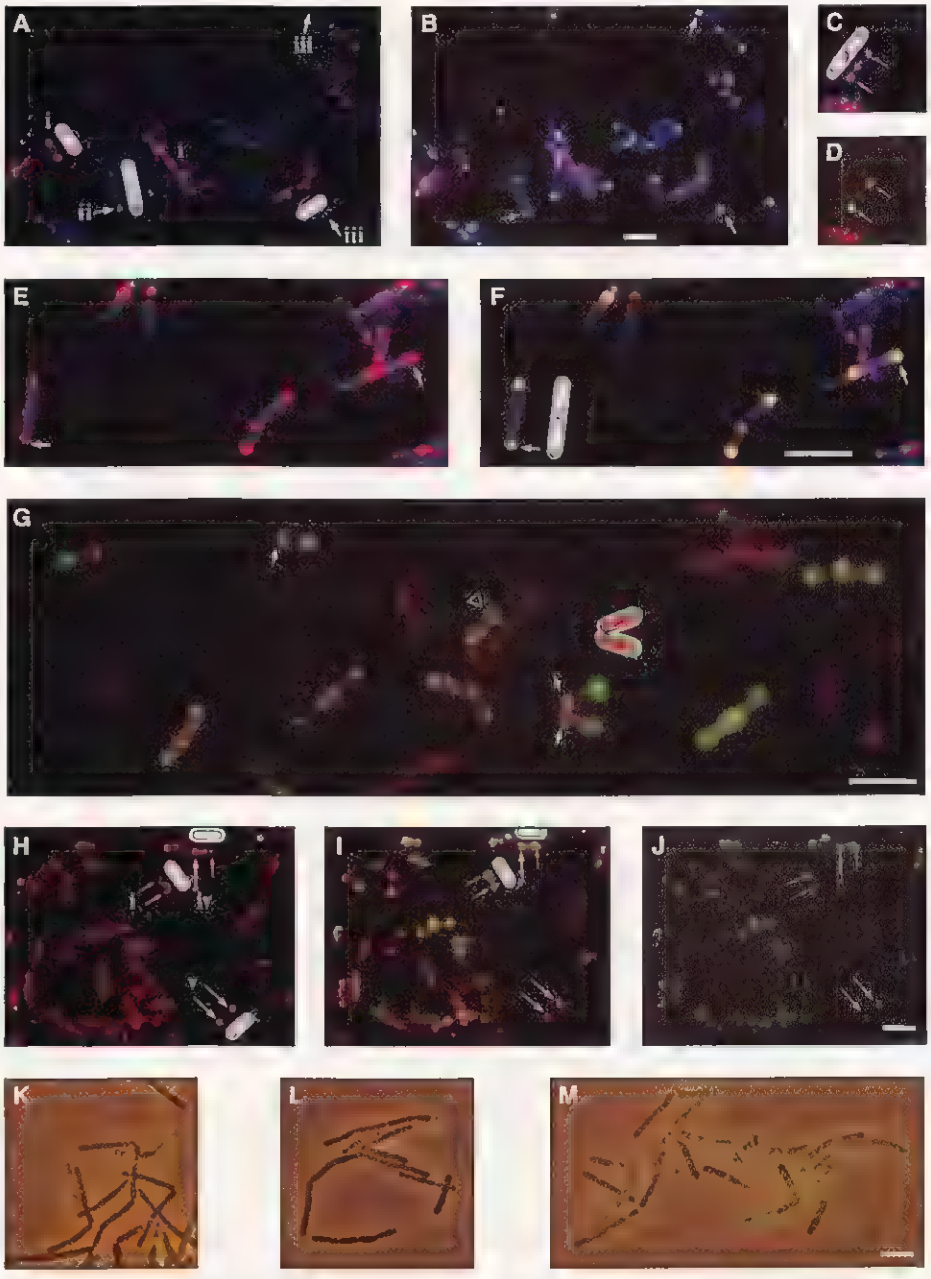

Fig. 1. Localization of SpoIIE in sporangia by fluorescence microscopy (*23*). (A to D) Immunolocalization (*24*) of SpoIIE (with Cy3-conjugated secondary antibodies) and β-Gal (with fluorescein-conjugated secondary antibodies) produced under the control of σ^F (*25*). (**A**) Doubly exposed micrograph showing immunostaining of SpoIIE (red) and DAPI staining of DNA (blue) (*13*). (**B**) Triply exposed micrograph of the same field with immunostaining of β-Gal (green-blue). White-to-pink corresponds to regions in which SpoIIE (red) and β-Gal (green-blue) coincide. (**C**) Doubly exposed micrograph showing SpoIIE (red) and the DAPI-stained DNA (blue) in a pair of sporangia with adjoining mother cells. (**D**) Doubly exposed micrograph of the same field as in (C) showing SpoIIE (red) and β-Gal (green) (*22*). Yellow color corresponds to regions in which SpoIIE and β-Gal are nearly coincident. (E and F) Immunolocalization of SpoIIE (Cy3) and β-Gal (fluorescein) produced under the control of σ^F in *spoIIIE* mutant sporangia (*26*). (**E**) shows SpoIIE (red) and the DAPI-stained DNA (blue), and (**F**) additionally shows β-Gal (green) (*27*). Yellow color occurs where SpoIIE and β-Gal overlap. (**G**) Immunolocalization of SpoIIE (fluorescein) in propidium iodide–stained (red) *spoIIIE* mutant sporangia (*28*). (H to J) Immunolocalization of SpoIIE (fluorescein) in a disporic mutant (*spoIIGAB::erm*) (*29*). (**H**) Propidium iodide–stained DNA (red). (**I**) Multiply exposed micrograph showing SpoIIE (green) and DNA (red); when the two are in close proximity, yellow is apparent. (**J**) SpoIIE alone (green). (**K** to **M**) Phase and fluorescence micrographs of living cells producing SpoIIE-GFP (*18*) at hour 2 of sporulation (*30*).

of the cell very early in development, probably before a septum has formed. Then, when the septum forms and the chromosomes segregate, SpoIIE disappears from the forespore-distal pole but remains localized near the forespore pole (*15*). Finally, after a chromosome is fully packaged into the forespore and σ^F is maximally active, SpoIIE disappears entirely from the sporangium.

A brighter image was obtained when immunostaining was carried out in a mutant (*spoIIIE*) defective in chromosome segregation in which class i sporangia persisted longer (Fig. 1E). The *spoIIIE* gene product has been implicated in the process of translocating one chromosome into the forespore (*16*). In studies with the *spoIIIE* mutant, it was apparent that SpoIIE is not located at the extreme ends of sporangia but instead is slightly internal from the poles, at or near the sites of asymmetric septation. This result was seen in mutant sporangia that were additionally stained for β-Gal produced under the control of σ^F. Fluorescence from immunostaining of β-Gal (green color in Fig. 1F, representing the same field as in Fig. 1E) in some of the sporangia (identified with arrows) extended beyond the region of SpoIIE immunostaining (red color) toward the cell pole. The overlap in the staining pattern of SpoIIE and β-Gal produced a yellow color. Furthermore, in some of the sporangia (identified with arrows) stained both for DNA (red color in Fig. 1G) and SpoIIE (green color), fluorescence from DNA extended out beyond the zone of SpoIIE immunostaining. Similar results were obtained with wild-type sporangia. Thus, SpoIIE coalesces at sites near to, but not at the ends of, cells. We sometimes observed SpoIIE immunostaining in the shape of a ring in the *spoIIIE* mutant (open triangle; Fig. 1G). This finding suggests that SpoIIE assembles into a collar-like structure on the inside surface of the cell at or near sites of potential septum formation (*17*).

We also determined the subcellular localization of SpoIIE with an additional approach. We constructed a strain producing a fusion protein of SpoIIE with green fluorescent protein (GFP) of *Aequorea victoria* (*18*). GFP autocatalytically generates a fluorophore that exhibits green fluorescence in unfixed, living cells (*19*). Sporangia producing the SpoIIE-GFP fusion protein displayed fluorescence patterns (Fig. 1, K to M) similar to that observed with immunostaining. Furthermore, phase contrast and fluorescence microscopy demonstrated that the SpoIIE-GFP fusion was localized in a sharp zone close to, but set back from, the poles of the sporangia (Fig. 1, L and M).

Normally, asymmetric division occurs at only one pole. Nonetheless, both poles are capable of undergoing division, as inferred from the existence of mutants that divide at both ends of the sporangium (*7*). Bipolar division in these mutants produces aberrant, "disporic" sporangia with chromosome-containing forespores in which σ^F is active (*20*) at both poles, and produces an empty mother cell in the middle (Fig. 3). We examined the localization of SpoIIE in a disporic mutant (*spoIIG*) (Fig. 1, H to J, representing the same field of cells). A bipolar pattern of SpoIIE immunostaining was observed in class i sporangia. At later stages of development, a class of sporangia (designated class iv) was observed with strong immunostaining at the forespore-distal pole. In these class iv sporangia, a condensed chromosome was visible at one pole of the cell (Fig. 1H), and a strong SpoIIE signal was detected at the opposite pole (Fig. 1, I and J), the site at which a second septum was presumably forming. Such class iv sporangia were abundant (76%; Fig. 2) among mutant sporangia at the stage of a condensed forespore chromosome but uncommon (2%; Fig. 2) among wild-type sporangia. Later in development, when the disporic mutant phenotype was fully manifest (class v sporangia), condensed chromosomes were visible at both poles (Fig. 1H), but relatively little SpoIIE could be detected at either end of the sporangia (Fig. 1, I and J). Therefore, in disporic sporangia, SpoIIE localizes near both poles during early stages of development, persists near each division site until a septum is formed at each pole, and finally disappears entirely from the sporangia (Fig. 3).

These observations suggest that the cell envelope contains "marks" at medial and

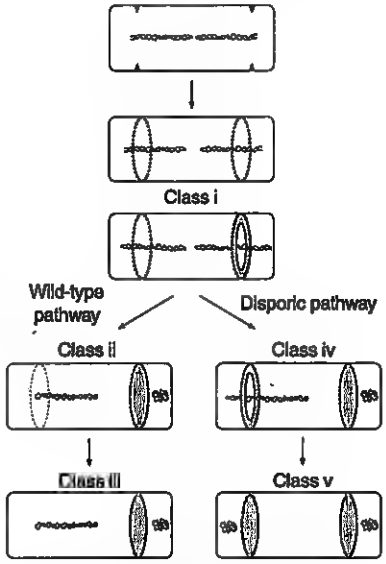

Fig. 3. Model for the subcellular localization of the cell-type determining protein SpoIIE in wild-type and disporic mutant bacteria. The cartoons represent cells at early stages of sporulation in wild-type and disporic mutant bacteria. The wavy horizontal lines indicate chromosomes; the chromosomes are extended when in the predivisional sporangium or the mother cell and compact when present in the forespore. The arrowheads in the top cartoon signify hypothetical marks for potential polar division. The incomplete, shaded disks represent still-forming septa, and the complete, shaded disks represent completed septa. The dotted oval lines represent SpoIIE, with the thin dotted lines indicating the presence of SpoIIE in low abundance and the dark dotted line indicating high abundance. In the wild-type pathway (on the left) a division septum is formed at only one pole, whereas in the disporic pathway of mutant bacteria, septa for m successively at both poles.

Fig. 2. Tabulation of SpoIIE immunostaining pattern in wild-type and in a disporic mutant. Immunostaining and DNA staining were done as described in Fig. 1 legend. Micrographs of the stained sporangia were scored according to the pattern of chromosome condensation and the pattern of SpoIIE localization. The percentages indicate the percentage of wild-type and disporic (*spoIIGAB::erm*) mutant sporangia exhibiting (right-hand column of percentages) or not exhibiting (left-hand column) a condensed forespore chromosome with the indicated pattern of SpoIIE immunostaining. The figure distinguishes two kinds of SpoIIE patterns: strong staining at both poles and preferential staining at one pole. [Note that for sporangia without a condensed forespore chromosome (left-hand column), the future forespore pole could not be distinguished from the future distal pole.] The percentages are based on 225 and 218 wild-type sporangia with or without a condensed chromosome, respectively, and 61 and 77 mutant sporangia with or without a condensed chromosome, respectively. Not included are wild-type sporangia that had reached the stage of development (when σ^F was fully active) at which little or no immunostaining could be detected. Also not included are disporic mutant sporangia that had reached the stage at which condensed, forespore chromosomes were present at both poles. Such sporangia in which the mutant phenotype was fully manifest (corresponding to class v in the text) displayed little immunostaining or weak staining, often preferentially at one pole.

SpoIIE pattern	Chromosome pattern	
	Wild type	
	68% class i	12%
	32%	86% class ii
		2%
	Disporic mutant	
	83% class i	16%
	17%	8%
		76% class iv

polar positions, and that at the start of sporulation the medial mark is masked or destroyed whereas marks at both poles are created or rendered accessible to the septation machinery of the cell (Fig. 3). The finding that SpoIIE initially localizes in the vicinity of both sites of potential polar division suggests that SpoIIE recognizes the hypothetical polar marks (Fig. 3) and hence could serve as a tool for verifying their existence and determining their molecular nature.

Finally, the finding that SpoIIE comes to be sequestered at the position of the polar septum indicates that SpoIIE sits at the boundary between the small cell in which σ^F is active and the large cell in which it is inactive. A simple model for how the septal location of SpoIIE could contribute to the selective activation of σ^F in the forespore, and the assignment of a biochemical function to SpoIIE, are presented in the accompanying report (9).

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18. The *spoIIE-gfp* fusion was constructed by polymerase chain reaction (PCR)–mediated amplification of *spoIIE* sequences from chromosomal DNA. The amplified DNA was cut at a Sac I site within the gene [located 1375 base pairs (bp) downstream of the start codon] and at an artificial Bam HI site located beyond the 3′ end of the open reading frame that had been created by a primer used in the PCR. This 1.1-kb Sac I–Bam HI fragment was cloned into the polylinker region located just upstream of *gfp* in plasmid pCW8 [C. D. Webb, A. Decatur, A. Teleman, R. Losick, *J. Bacteriol.*, in press]. This created pCW27, which contained the 3′ half of *spoIIE* joined in-frame to *gfp* by means of 13 codons of polylinker sequence. Next, a kanamycin-resistance gene was inserted in pCW27 to create pCW28 which was, in turn, integrated into the chromosomal *spoIIE* locus of wild-type strain PY79 by single, reciprocal (Campbell-like) recombination to create strain CW314. Integration of pCW28 created in the chromosome an intact *spoIIE* ORF that was joined in-frame to *gfp*. CW314 sporulates at nearly wild-type levels.
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21. KJP86 [*amyE::sspE(2G)-lacZ, spoIIIGΔ1*] is a derivative of strain PY79. *sspE(2G)* is a modified promoter that is recognized by σ^F-RNA polymerase [D. Sun *et al.*, *J. Bacteriol.* **173**, 7867 (1991)]. Similar results were obtained without *spoIIIGΔ1*, which inactivates σ^G, a sigma factor that also recognizes the *sspE(2G)* promoter.
22. The upper sporangium of the pair is an example of a class ii sporangium (it has unipolar SpoIIE and β-Gal), whereas the lower sporangium is intermediate between class i (it has bipolar SpoIIE) and class ii (it also has β-Gal).
23. The sporangia are frequently in chains or pairs with the mother cells adjoining one another. The scale bars are 5 μm. Sporulation was induced by the resuspension method [J. M. Sterlini and J. Mandelstam, *Biochem. J.* **113**, 29 (1969)]. Arrows in (A to F) point to forespores. The interpretative cartoons in (A, C, F, G, and I) illustrate the pattern of immunostaining of SpoIIE or β-Gal or both.
24. Immunofluorescence microscopy was done as described (*11*) with the following modifications. The bacteria were fixed with a final concentration of 2.6% paraformaldehyde and either 0.005% or 0.013% glutaradehyde, directly in resuspension medium, to which sodium phosphate buffer (pH 7.5) was added to a final concentration of 30 mM. The methanol/acetone treatment was omitted, and the slides were incubated at 4°C overnight with the primary antibodies. Antibodies to SpoIIE were produced in rabbits, affinity-purified as described (*10*), and used at a 1:5 dilution. Mouse antibodies directed against β-Gal were obtained from Promega and used at a 1:1500 dilution. For the simultaneous localization of SpoIIE and β-Gal in Fig. 1, A to F, the slides were incubated overnight in a mixture of these two antibodies, washed in phosphate-buffered saline (PBS), and then incubated for ~2 hours in a solution containing DAPI (0.2 mg/ml), and a mixture of two secondary antibodies (both at 0.75 mg/ml)—multiple-labeling grade, Cy3-conjugated goat antibodies to rabbit immunoglobulin G (IgG), and multiple-labeling grade, fluorescein-conjugated goat antibodies to mouse IgG (Jackson ImmunoResearch Laboratories). When only SpoIIE was localized (Fig. 1G), the slides were incubated overnight with antibodies to SpoIIE, washed, and incubated in a solution of fluorescein-conjugated goat antibodies to rabbit IgG (0.75 mg/ml) and propidium iodide (0.1 mg/ml). The samples were then washed with PBS containing propidium iodide (0.1 mg/ml).
25. Cells of strain KJP86, which contains *lacZ* fused to a promoter under the control of σ^F (*21*), were collected either 3 hours (A and B) or 2 hours (C and D) after the start of sporulation. Class i sporangia have bipolar staining of SpoIIE and no detectable β-Gal (examples of a single sporangium and a chain of four sporangia are indicated); class ii stain weakly for both SpoIIE and β-Gal (one forespore of a pair of sporangia is indicated); class iii have little or no detectable SpoIIE but stain strongly for β-Gal (two single sporangia are identified).
26. Cells of strain KJP123 [*spoIIIE::spc, amyE::sspE(2G)-lacZ, spoIIIGΔ1*], which contains a *spoIIIE::spc* null mutation (constructed by P. A. Levin) and *lacZ* fused to a promoter under the control of σ^F (*22*) were collected 2 hours after the start of sporulation (*23*) and processed for immunofluorescence microscopy (*24*).
27. In the two sporangia identified by arrows, β-Gal extends beyond SpoIIE, as demonstrated by the green region on the mother cell–distal side of the forespore compartment and the red region on the mother cell–proximal side of the forespore.
28. Cells of strain PL412 (*spoIIIE::spc*) were collected 2 hours after the start of sporulation and processed for immunofluorescence. The arrows indicate sporangia in which the propidium iodide–stained DNA (red) projects beyond SpoIIE (green). The open triangle indicates a sporangium with a ring of SpoIIE.
29. Indicated are a class i sporangium in which chromosome segregation is incomplete; a class iv disporic sporangium in which one forespore contains a completely segregated chromosome but the second does not; and a class v sporangium in which both forespore compartments contain a fully segregated chromosome. The long arrows point to forespores with a fully segregated chromosome, and the short arrows point to forespores with an incompletely segregated chromosome. The interpretative cartoons in (H) signify the presence of a forespore with a fully segregated chromosome (as inferred from the pattern of DNA staining) by a septal crossline.
30. The arrows point to two sporangia in which the forespore pole extends beyond SpoIIE-GFP. (M) contains many chains of sporangia; the circular pattern apparent in such cells results from the adjacent crescents of SpoIIE-GFP in neighboring sporangia, not from a SpoIIE ring in a single sporangium.

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Activation of Cell-Specific Transcription by a Serine Phosphatase at the Site of Asymmetric Division

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Cell fate is determined by cell-specific activation of transcription factor σ^F after asymmetric division during sporulation by *Bacillus subtilis*. The activity of σ^F is governed by SpoIIAA, SpoIIAB, and SpoIIE, a membrane protein localized at the polar septum. SpoIIAB binds to and inhibits σ^F, and SpoIIAA inhibits SpoIIAB, which prevents SpoIIAB from binding to σ^F. SpoIIAB is also a serine kinase that inactivates SpoIIAA. Here, it is demonstrated that SpoIIE dephosphorylates SpoIIAA-P and overcomes SpoIIAB-mediated inhibition of σ^F. The finding that SpoIIE is a serine phosphatase links asymmetric division to the pathway governing cell-specific gene transcription.

The σ^F factor of the bacterium *Bacillus subtilis* is a transcriptional control protein that is required for the establishment of cell-specific gene expression during the process of sporulation (*1*). During sporulation, a polar septum forms that partitions the developing cell or sporangium into two unequal cellular compartments, the forespore and the mother cell. The σ^F factor is present before the formation of the polar septum and is activated in the forespore after septation. An important challenge is to understand the events that lead to the activation of σ^F in one cellular compartment. The activity of σ^F is governed by three regulatory proteins: SpoIIE, SpoIIAA, and SpoIIAB. Epistasis experiments indicate that these proteins constitute a regulatory hierarchy in which SpoIIE is an activator of SpoIIAA, SpoIIAA is an inhibitor of SpoIIAB, and SpoIIAB is an inhibitor of σ^F (*2*). Consistent with these relations, biochemical evidence indicates that SpoIIAB is an anti-sigma factor that binds to σ^F and holds it in an inactive complex and that SpoIIAA is an anti–anti-sigma factor that binds to SpoIIAB, thereby preventing it from binding to σ^F (*3–6*). SpoIIE is inferred to be an integral membrane protein with 8 to 10 membrane-spanning segments in the NH_2-terminal domain (residues 1 to 322) and with a large cytoplasmic domain in the COOH-terminus (residues 323 to 827) (*7*). SpoIIE becomes localized during division to the polar septum, which separates the forespore from the mother cell (*8*). However, the function of SpoIIE has been unknown (*2*).

SpoIIAA and SpoIIAB govern the activity of σ^F by a partner-switching mechanism in which the SpoIIAB·σ^F complex exchanges σ^F for SpoIIAA to release free and active σ^F (*4, 5, 9*). Partner switching is strongly influenced by the adenosine nucleotides adenosine triphosphate (ATP) and adenosine diphosphate (ADP) in vitro. Thus, ADP stimulates the formation of the SpoIIAB·SpoIIAA complex and favors the release of σ^F. Conversely, ATP stimulates the formation of the SpoIIAB·σ^F complex and thus causes inhibition of σ^F (*4, 5*). In addition, SpoIIAB is a protein kinase that uses ATP to phosphorylate SpoIIAA on Ser^{58} (*6, 10*), which inactivates SpoIIAA and prevents it from binding to SpoIIAB (*4, 5*).

To investigate the function of SpoIIE, a fusion protein consisting of the 505-residue COOH-terminal domain (residues 323 to 827) of SpoIIE joined to six histidine residues (hereafter called $SpoIIE_{323\text{-}827}$) was purified from *Escherichia coli* by nickel affinity chromatography (*11*) and incubated with SpoIIAA-P (where *P* indicates phospho-SpoIIAA) that was labeled with [^{35}S]methionine (Fig. 1A). Analysis of the products of the reaction by isoelectric focusing demonstrated that increasing concentrations of $SpoIIE_{323\text{-}827}$ converted ^{35}S-labeled SpoIIAA-P (whose isoelectric point was identical to that of SpoIIAA-P which had been labeled with ^{32}P) to a form whose isoelectric point was identical to that of unphosphorylated SpoIIAA (Fig. 1A, lanes 1 to 8). $SpoIIE_{323\text{-}827}$ also caused the loss of radioactivity from SpoIIAA-P that was labeled with ^{32}P, which confirmed that $SpoIIE_{323\text{-}827}$ is a serine phosphatase that had removed the phosphate residue from SpoIIAA-P (Fig. 1C) (*12*). The serine phosphatase activity of SpoIIE was greatly stimulated by Mn^{2+} (Fig. 1C), a cofactor for certain serine-threonine phosphatases (*13*). As a control for specificity, $SpoIIE_{323\text{-}827}$ failed to dephosphorylate the phosphorylated form of RsbV, a homolog of SpoIIAA that controls the activity of the *B. subtilis* stress response sigma factor σ^B (Fig. 1B) (*14, 15*).

An additional control that provided physiological evidence for the function of the phosphatase activity came from the use of a mutant form of SpoIIAA in which Ser^{58} (the site of phosphorylation) was replaced with Thr (S58T). Cells producing the SpoIIAA-S58T mutant protein are blocked in the induction of σ^F-directed gene transcription (*9*). When incubated with SpoIIAB and ATP, the mutant protein readily underwent phosphorylation (*16*) (compare lanes 9 and 11 in Fig. 1A). However, incubation of the phosphorylated mutant protein with $SpoIIE_{323\text{-}827}$ failed to convert it back to the dephosphorylated form (Fig. 1A, lanes 11 and 12). Evidently, SpoIIAA-S58T is a substrate for the kinase but not for the phosphatase. This finding

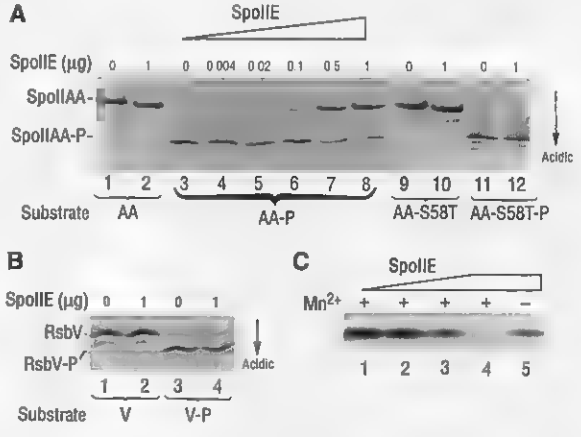

Fig. 1. The cytoplasmic domain of SpoIIE is a serine phosphatase. (**A** and **B**) Autoradiographs of the products of reactions containing phosphorylated or unphosphorylated SpoIIAA, SpoIIAA-S58T, or RsbV incubated with $SpoIIE_{323\text{-}827}$ and separated by isoelectric focusing. SpoIIAA, SpoIIAA-S58T, and RsbV were labeled with [^{35}S]methionine and phosphorylated as described (*22*). Next, the phosphorylated or unphosphorylated proteins were incubated for 30 min at 30°C with or without $SpoIIE_{323\text{-}827}$ in 40-μl reactions containing 2 mM $MnCl_2$. The reactions were then stopped by the addition of an equal volume of loading solution (*23*). The products of the reaction were separated on a 5% polyacrylamide isoelectric focusing slab gel (*23*). (**C**) Autoradiograph of the products of dephosphophorylation reactions containing ^{32}P-labeled SpoIIAA-P and various quantities of $SpoIIE_{323\text{-}827}$. ^{32}P-labeled SpoIIAA-P (*24*) was incubated in 50 μl of buffer A (*24*) in the presence or absence of 2 mM $MnCl_2$ (as indicated) and the following quantities of $SpoIIE_{323\text{-}827}$ for 30 min at 30°C: lane 1, 0 μg; lane 2, 0.2 μg; lane 3, 1 μg; and lanes 4 and 5, 5 μg. Reactions were terminated by the addition of SDS-polyacrylamide gel sample buffer. The reaction products were separated in an 18% SDS-polyacrylamide slab gel, which was fixed and dried.

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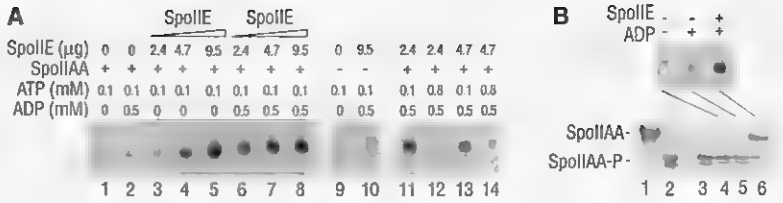

Fig. 2. $SpoIIE_{323-827}$ overcomes SpoIIAB-mediated inhibition of σ^F. (**A**) Autoradiographs of the products of run-off transcription reactions that had been subjected to electrophoresis in urea–8% polyacrylamide sequencing gels. Reaction mixtures (*25*) containing σ^F, SpoIIAB, 100 μM ATP, template DNA, and SpoIIAA (where indicated) were incubated for 10 min at 37°C. Next, aliquots (100 μl) were removed and supplemented with ADP, additional ATP, and $SpoIIE_{323-827}$ as indicated. Finally, transcription was carried out by the addition of core RNA polymerase (*21*). (**B**) The phosphorylation state of SpoIIAA correlates with the activity of σ^F. The top shows the products of σ^F directed transcription, and the bottom shows the phosphorylation state of SpoIIAA from the transcription reaction mixtures. A reaction mixture containing σ^F, SpoIIAB, SpoIIAA, 100 μM ATP, and template DNA was incubated for 10 min at 37°C (*26*). Next, an aliquot (100 μl) was removed for isoelectric focusing (lane 3). Additional aliquots (200 μl) were removed and mixed with either no further components (lane 4), with 500 μM ADP (final concentration) (lane 5), or with 500 μM ADP (final concentration) and 38 μg of $SpoIIE_{323-827}$ (lane 6). After 5 min of incubation at 37°C, half (100 μl) of each of these reaction mixtures was removed and samples (containing about 260 ng of SpoIIAA) were subjected to isoelectric focusing (*23*) and protein immunoblot analysis with SpoIIAA antibodies (*9*). Transcription was then carried out in the remainder of each reaction mixture by the addition of core RNA polymerase (*21*). As isoelectric focusing markers, 380 ng of SpoIIAA (lane 1) and 380 ng of SpoIIAA-P (lane 2) were subjected to isoelectric focusing and protein immunoblot analysis.

explains the phenotype of cells that produce the mutant protein: SpoIIAA-S58T-P cannot undergo dephosphorylation in vivo and hence cannot overcome SpoIIAB-mediated inhibition of σ^F.

Next, we investigated whether $SpoIIE_{323-827}$ could reverse the SpoIIAB-mediated inhibition of σ^F-directed transcription. SpoIIAA, SpoIIAB, σ^F, and template DNA were incubated with 100 μM ATP under conditions that inactivate SpoIIAA by SpoIIAB-mediated phosphorylation and allow the formation of SpoIIAB·σ^F complexes (*9*). Transcription was initiated by the addition of core RNA polymerase in the presence or absence of $SpoIIE_{323-827}$. $SpoIIE_{323-827}$ effectively restored σ^F-directed RNA synthesis in the presence of SpoIIAB (Fig. 2A, lanes 1 through 8), and this restoration depended on the presence of SpoIIAA (Fig. 2A, compare lanes 8 and 10). Moreover, consistent with previous findings that ADP stimulates the formation of SpoIIAB·SpoIIAA complexes, the effect of 2.4 μg of $SpoIIE_{323-827}$ was strongly enhanced by the presence of 500 μM ADP (compare lanes 3 and 6 of Fig. 2A). (However, at higher concentrations $SpoIIE_{323-827}$ effectively restored transcription in the absence of added ADP; for example, compare lanes 5 and 8 of Fig. 2A.) Conversely, increasing the concentration of ATP from 100 μM to 800 μM, even in the presence of 500 μM ADP and 2.4 or 4.7 μg of $SpoIIE_{323-827}$, substantially prevented the restoration of σ^F-directed transcription (Fig. 2A, lanes 11 through 14).

Finally, to confirm that the effect of $SpoIIE_{323-827}$ was exerted at the level of dephosphorylation of SpoIIAA-P, isoelectric focusing and protein immunoblot analysis were performed to determine the state of phosphorylation of SpoIIAA in transcription reaction mixtures. In the absence of $SpoIIE_{323-827}$, SpoIIAA was principally present in the phosphorylated state, and in the presence of $SpoIIE_{323-827}$ SpoIIAA was largely unphosphorylated (Fig. 2B). Thus, the transcriptional activity of σ^F was correlated with the phosphorylation state of SpoIIAA.

The COOH-terminal region of SpoIIE and its homolog in *Bacillus megaterium* are similar to the COOH-terminal region of the product (RsbU) (*17*) of a regulatory gene in the pathway governing the activation of a stress response transcription factor (σ^B) in *B. subtilis* and marginally similar to the COOH-terminal region of another compo-

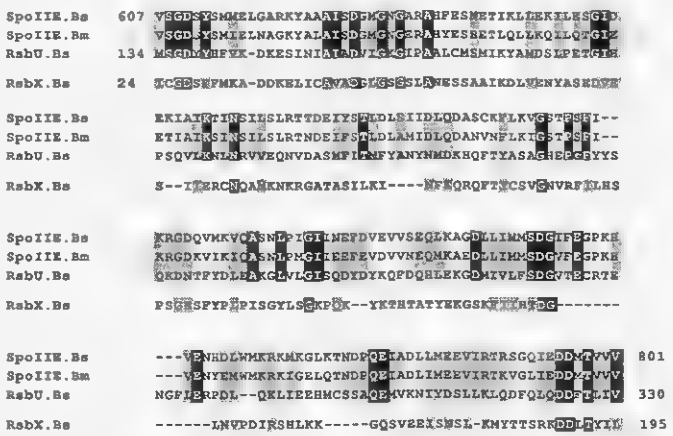

Fig. 3. A family of serine phosphatases Shown is an alignment of the cytoplasmic domain of SpoIIE from *B. subtilis* (SpoIIE.Bs) (*20*) and from *B. megaterium* (SpoIIE.Bm; GenBank accession number U26836) with regions of RsbU (RsbU.Bs) (*17*) and RsbX (RsbX.Bs) (*14*) from *B. subtilis*. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu, M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. Conservative amino acid substitutions between SpoIIE.Bs, SpoIIE.Bm, and RsbU.Bs are highlighted in gray, and identities are in black (*27*). Because of its weaker similarity, RsbX.Bs is presented separately. Amino acid sequence similarity was also noted (not shown) to a protein (IcfG; protein identification resource accession number S38573) of unknown function from *Synechocystis*. The numbers indicate the positions of the residues in each of the proteins; in the case of SpoIIE.Bm, the full predicted sequence is not available and hence the numbers are not indicated.

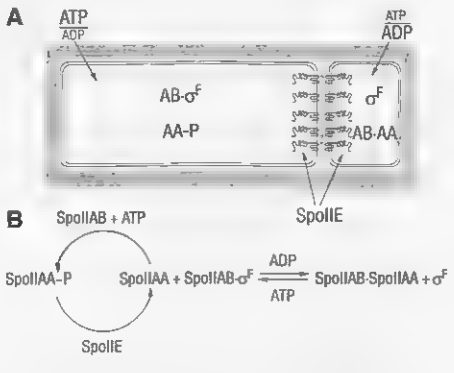

Fig. 4. A model for the establishment of cell-specific gene transcription. (**A**) The COOH-terminal (serine phosphatase) domain (represented by the curlicues) of SpoIIE could be more concentrated in the forespore (the small cell) than in the mother cell by virtue of being displayed on the cytoplasmic side of the membranes on either side of the division septum. The NH_2-terminal domain is represented by the series of transmembrane segments. The stippled material represents the cell wall, and the parallel double lines represent the cytoplasmic membranes. (**B**) The pathway extending from SpoIIE to the activation of σ^F. The phosphorylation state of SpoIIAA is governed by a cycle of opposing kinase (SpoIIAB) and phosphatase (SpoIIE) activities. Dephosphorylated SpoIIAA displaces σ^F from the SpoIIAB·σ^F complex to yield SpoIIAB·SpoIIAA and free and active σ^F. ADP and ATP oppositely influence partner switching and hence indirectly govern phosphorylation by determining whether the SpoIIAB kinase is sequestered in an inactive complex with SpoIIAA. Activation of σ^F could be augmented by a selective decrease in the ratio of ATP to ADP in the forespore as depicted in (A) or by an overall drop in the ratio of ATP to ADP in the sporangium.

nent (RsbX) (*14*) of the σ^B regulatory system (Fig. 3). The activity of σ^B is controlled by homologs of SpoIIAA and SpoIIAB called RsbV and RsbW, respectively (*14*, *15*). RsbV is an anti–anti-sigma factor that binds to RsbW, and RsbW is an anti-sigma factor of σ^B and also a protein kinase that phosphorylates RsbV (*15*). RsbU and RsbX may be serine phosphatases that dephosphorylate RsbV-P or the phosphorylated form of an additional related protein in the σ^B system encoded by *orfS* (*17*).

Combined with other results (*3*–*6*, *8*), the discovery that the COOH-terminal region of SpoIIE is a serine phosphatase allows the assignment of a function for each step in the pathway that extends from asymmetric division to cell-specific activation of σ^F. During the partitioning of the sporangium into two cellular compartments, SpoIIE localizes to the polar septum (Fig. 4A), where its COOH-terminal cytoplasmic domain activates SpoIIAA-P by dephosphorylation (Fig. 4B). Next, dephosphorylated SpoIIAA interacts with the SpoIIAB·σ^F complex, which causes an exchange of partners that results in the formation of the SpoIIAB·SpoIIAA complex and the release of free and active σ^F (Fig. 4B). If the cytoplasmic domain of SpoIIE is displayed equally on the forespore and mother-cell sides of the polar septum, then the phosphatase would be more concentrated in the forespore than in the mother cell because of the smaller volume (no more than one-fifth as large) of the former (Fig. 4A). This higher effective concentration of phosphatase would result in more σ^F activity in the forespore than in the mother cell; however, this may be insufficient to account for the strict cell-specific activity of σ^F (*18*). In confirmation and extension of previous results showing that partner switching by, and the kinase activity of, SpoIIAB is strongly influenced by ADP and ATP (*4*, *5*), the capacity of the SpoIIE phosphatase to counteract the effect of the SpoIIAB kinase was partially dependent on ADP and strongly inhibited by ATP. Thus, the cell-specific activation of σ^F could be a composite consequence of the septal location of SpoIIE and an alteration in cellular adenosine nucleotide levels (Fig. 4A) (*19*).

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12. Silver staining confirmed that the loss of radioactivity from ^{32}P-labeled SpoIIAA-P was not due to SpoIIE$_{323-827}$-induced degradation of SpoIIAA. Substantial dephosphorylation required approximately stoichiometric amounts of SpoIIE$_{323-827}$ and SpoIIAA, perhaps indicating that SpoIIE$_{323-827}$ was inefficiently renatured in our protocol (*11*) or that the NH_2-terminal, integral membrane portion of the protein is needed for full activity. Alternatively, SpoIIE may normally be present and act in roughly equimolar concentration to that of SpoIIAA during sporulation. Strictly speaking, these results do not distinguish between SpoIIE being the phosphatase or an activator of a SpoIIAA autophosphatase. For simplicity, the former is assumed to be the case. Consistent with the idea that SpoIIE is the phosphatase, SpoIIAA-P was highly stable and little or no dephosphorylation was observed in the absence of SpoIIE$_{323-827}$. The phosphate moiety of SpoIIAA-P is evidently liberated by hydrolysis because little or no ^{32}P was transferred to an acid-stable (hydroxyl) moiety on SpoIIE$_{323-827}$.
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16. Phosphorylation presumably occurred at Thr^{58} because an Ala^{58} substitution mutant does not undergo phosphorylation (*5*), but conceivably the S58T mutant undergoes phosphorylation at a serine residue in the vicinity of amino acid 58.
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19. Alternatively, cell-specific activation of σ^F could be governed exclusively by the SpoIIE phosphatase if, by an unknown mechanism, the phosphatase activity is restricted to the forespore face of the septum.
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21. Transcription buffer contained 40 mM tris-HCl (pH 8.0), 50 mM KCl, 10 mM $MgCl_2$, 2 mM $MnCl_2$, 0.4 mM DTT, 200 μM guanosine triphosphate, 200 μM uridine triphosphate, 0.02 μM unlabeled cytosine triphosphate (CTP), and 0.1 μCi/μl of [α-^{32}P]CTP (10 μCi/μl, 3.3 μM; NEN). Transcription reactions were initiated by the addition of 200 ng of *B. subtilis* core RNA polymerase (*3*). Purification of σ^F was as described (*9*). The template for transcription was pLD14 that had been linearized with Hinc II, which yielded run-off transcripts of 87 nucleotides (*9*). pLD14 contains a strong σ^F-dependent promoter [*sspE-2G*; D. Sun *et al.*, *J. Bacteriol.* **173**, 7867 (1991)] fused upstream of DNA lacking adenosine nucleotides on the nontemplate strand (*9*). Transcription reactions were terminated after 15 min at 37°C by the addition of heparin (500 μg/ml, final concentration) and unlabeled CTP (200 μM, final concentration). After incubation for 5 min at 37°C, RNA in the reaction mixtures was precipitated twice with 3 volumes of ethanol/3 M sodium acetate [40:1 (v/v)] in the presence of 13.5 μg of carrier yeast RNA, dried, and resuspended in 10 μl of formamide loading solution. Samples were boiled and loaded on urea–8% polyacrylamide sequencing gels.
22. *spoIIAA*, *spoIIAA-S58T*, and *rsbV* were expressed in *E. coli* with an IPTG-inducible T7 RNA polymerase system, and the protein products were labeled with [^{35}S]methionine as described (*4*). The *spoIIAA* expression plasmid and strain have been described (*4*). The construction of the *spoIIAA-S58T* expression plasmid (containing a mutant *spoIIAA* in which Ser codon 58 was converted to a Thr codon) and the construction of the *rsbV* T7-RNA polymerase expression plasmid and strain were as described (*9*). To generate ^{35}S-labeled SpoIIAA-P and ^{35}S-labeled SpoIIAA-S58T-P, cell pellets of the expression strains corresponding to 2 ml of [^{35}S]methionine–labeled cells were resuspended in 1 ml of lysis buffer (*9*) and the cells lysed by freeze-thawing. Insoluble debris was removed by centrifugation for 10 min at 4°C. ATP was added to a final concentration of 1 mM, and 200 μl of the extract was passaged five times over a 20-μl column containing 4 μg of purified SpoIIAB immobilized on a solid support (Affi-Gel-10; Bio-Rad) (*9*). This procedure generates quantitatively phosphorylated ^{35}S-labeled SpoIIAA and SpoIIAA-S58T free of SpoIIAB. This ^{35}S-labeled SpoIIAA-P and SpoIIAA-S58T-P (20 μl each) was used in each dephosphorylation experiment in Fig. 1A. ^{35}S-labeled RsbV-P was generated in a similar manner with the use of purified RsbW, except that the ^{35}S-labeled RsbV was passaged five times over a 20-μl column containing approximately 4 μg of RsbW immobilized on Affi-Gel-10 (*9*). As verification of phosphorylation, ^{35}S-labeled SpoIIAA-P and ^{35}S-labeled RsbV-P had isoelectric points identical to those of the corresponding ^{32}P-labeled proteins.
23. The loading solution for isoelectric focusing contained 8 M urea, 12% ampholytes (pH 3 to 10) (Pharmalyte; Pharmacia), 2% Triton X-100, 1% 2-mercaptoethanol, and 0.1% bromophenol blue. The 5% polyacrylamide isoelectric focusing slab gels contained 2.5% (w/v) ampholytes (pH 3 to 10) (Pharmalyte; Pharmacia) and 8 M urea and were run for 2 hours at 300 V. The anolyte was 10 mM phosphoric acid, and the catholyte was 20 mM NaOH. The gels of Fig. 1, A and B, were fixed for 10 min in 10% trichloroacetic acid, rinsed in water, and dried. The weaker radioactive species are isoelectric variants of SpoIIAA and RsbV that presumably arose by deamination or other protein modification during isolation. The gel of the bottom of Fig. 2B was soaked in transfer buffer (48 mM tris base, 39 mM glycine, 0.037% SDS, and 20% methanol) for 30 min before transfer to Immobilon-P membrane (Millipore) and subjected to protein immunoblotting (*9*).
24. Purification of SpoIIAA and SpoIIAB was as described (*9*). Purified SpoIIAA (6 μg) was phosphorylated in 100 μl of reaction mixture containing 20 μg of SpoIIAB immobilized on Affi-Gel-10 (Bio-Rad) (*9*) and 10 μCi of [γ-^{32}P]ATP (New England Nuclear) in buffer A [20 mM Hepes (pH 7.5), 100 mM NaCl, 10% glycerol, 10 mM $MgCl_2$, 1 mM DTT, 50 μM ATP] for 30 min at room temperature. SpoIIAB was removed by centrifugation, and 5-μl aliquots of SpoIIAA-^{32}P (corresponding to approximately 300 ng of protein) were used in the dephosphorylation experiments of Fig. 1C.
25. Reaction mixtures contained 3 μg of σ^F, 4 μg of SpoIIAB, 17.6 μg of SpoIIAA, 100 μM ATP, and 20 μg of template in 1 ml of transcription buffer (*21*) (lanes 1 to 8); or 1.2 μg of σ^F, 1.6 μg of SpoIIAB, 100 μM ATP, and 8 μg of template in 400 μl of buffer (lanes 9 and 10); or 3 μg of σ^F, 4 μg of SpoIIAB, 17.6 μg of SpoIIAA, 100 μM ATP, and 20 μg of template in 1 ml of buffer (lanes 11 to 14).
26. The reaction mixture contained 2.4 μg of σ^F, 3.2 μg

of SpoIIAB, 14.1 μg of SpoIIAA, 100 μM ATP, and 16 μg of template in transcription buffer (800 μl) (*21*).
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Central Command Neurons of the Sympathetic Nervous System: Basis of the Fight-or-Flight Response

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During stress, the activity of the sympathetic nervous system is changed in a global fashion, leading to an increase in cardiovascular function and a release of adrenal catecholamines. This response is thought to be regulated by a common set of brain neurons that provide a dual input to the sympathetic preganglionic neurons regulating cardiac and adrenal medullary functions. By using a double-virus transneuronal labeling technique, the existence of such a set of central autonomic neurons in the hypothalamus and brainstem was demonstrated. These neurons innervate both of the sympathetic outflow systems and likely function in circumstances where parallel sympathetic processing occurs, such as in the fight-or-flight response.

The sympathetic nervous system regulates a broad range of visceral functions and, during extreme emotional or physical states, activates both the cardiovascular and adrenal catecholamine systems for homeostatic adjustments (*1*). The central nervous system (CNS) neurons responsible for coactivation of these autonomic changes are thought to be governed by a common set of central command neurons that provides dual projections to the sympathetic outflow systems that control the heart and adrenal gland. Although this biological idea was described in the late 1920s (*1*) and is taught as a basic principle of autonomic function, it has not been possible to define the command neurons and CNS circuits responsible for this response, because of the technical limitations. We have now developed a double-virus transneuronal labeling method to localize and to chemically characterize the central command neurons.

The general scheme of this study is presented in Fig. 1A. Two different genetically engineered forms of the Bartha strain of pseudorabies virus (PRV) were used as transneuronal tracers (*2*); each expressed a unique marker antigen in infected host cells (Fig. 1B). Both produce specific infections within functionally related chains of neurons. One virus was injected into the stellate ganglion—the major sympathetic ganglion that innervates the heart (*3*)—and the other virus was injected into the ipsilateral adrenal gland of anesthetized Sprague-Dawley rats or vice versa (*4*). After 4 days, rats were anesthetized and perfused with fixative, and their brains processed by a triple-antibody immunohistochemical procedure for the two unique virally induced cellular markers (gC viral glycoprotein and β-galactosidase) and also stained for a neurotransmitter enzyme or neurotransmitter (choline acetyltransferase, phenylethanolamine-*N*-methyltransferase, tyrosine hydroxylase, serotonin, or oxytocin) (*5*). A total of 20 rats contained double-virus infections; eight of these had CNS patterns of infection for both viruses that were similar to those found in earlier studies in which a single strain of PRV was injected into the adrenal gland (*6*) or the stellate ganglion (*7*).

The brain sites that were transneuronally labeled with the two different viruses that had been injected into the terminal fields of the sympathoadrenal and stellate sympathetic preganglionic neurons are illustrated in Fig. 2. Three areas of the medulla oblongata were labeled: (i) rostral ventrolateral medulla; (ii) rostral ventromedial medulla, which includes the lateral paragigantocellular reticular nucleus, parapyramidal nucleus, and ventral and pars alpha regions of the gigantocellular reticular nuclei; and (iii) caudal raphe nuclei (raphe magnus, raphe pallidus, and raphe obscurus). Monoaminergic medullary neurons contribute to this projection. C1 adrenergic neurons (Fig. 3), in both the rostral

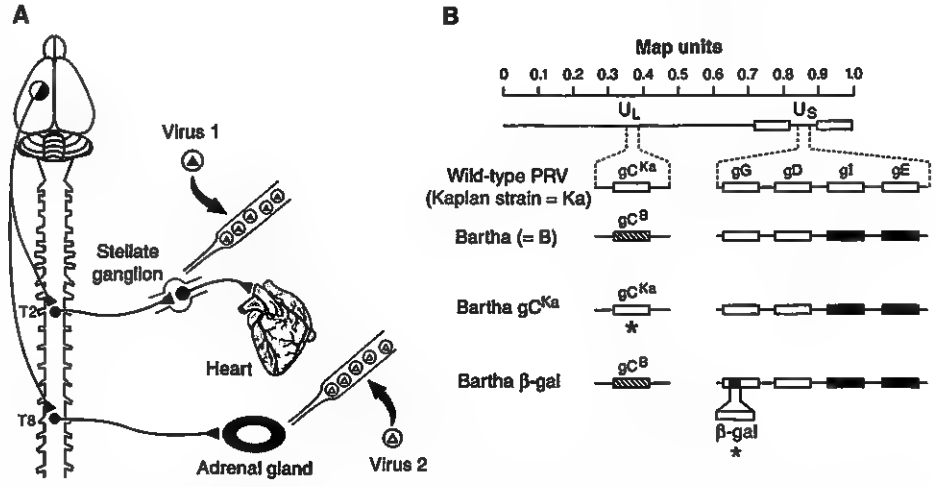

Fig. 1. (**A**) The CNS sites that regulate the sympathetic outflow of both the stellate ganglion and adrenal gland were identified by a double-virus transneuronal labeling method. In the same animal, one virus was injected into the stellate ganglion and the second virus into the ipsilateral adrenal gland. Each virus produced a unique intracellular marker in infected host neurons, and some neurons contained both markers, indicating that they regulate both sympathetic systems. (**B**) Two genetically modified forms of Bartha PRV used for transneuronal labeling of central sympathetic circuits (Bartha-gC^{Ka} PRV and Bartha β-galactosidase PRV). The genomes of these two viruses differ from the wild-type PRV and the original attenuated Bartha strain. Each modified virus contained a gene that produced a different intracellular antigen in the infected host neurons that could be detected by specific antibodies (asterisks). Bartha-gC^{Ka} PRV produced the wild-type form of the gC glycoprotein, which was detected by a mouse monoclonal antibody. Bartha β-galactosidase PRV was detected by a goat polyclonal antibody directed against β-galactosidase (*2*). U_L, unique long segment; U_S, unique short segment. Black boxes, deleted sequences; striped boxes, altered sequences.

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ventrolateral medulla and lateral paragigantocellular reticular nucleus, provided the greatest projection to the spinal cord and were most concentrated in the rostral ventrolateral medulla immediately caudal to the facial nucleus (Table 1). Double-labeled serotonergic neurons of the caudal raphe nuclei were found mainly in the parapyramidal and raphe magnus nuclei. In the pons, 25% of the infected A5 neurons were catecholaminergic, whereas none of the infected subcoeruleus neurons showed this property (Table 1). In three animals, the caudal ventrolateral periaqueductal gray matter (laterodorsal tegmental nucleus) contained double-labeled neurons, and most of these exhibited choline acetyltransferase immunoreactivity. In the hypothalamus, double-labeled neurons were found in the paraventricular and caudal lateral hypothalamic nuclei with additional neurons found in the perifornical area. Less than 10% of the double-infected neurons in the paraventricular hypothalamic nucleus contained oxytocin immunoreactivity. In the spinal cord, a small number of double-infected neurons were found in the dorsal horn (laminae I, II, and V) and intermediate gray matter (lamina VII) (approximately seven in an alternate series of sections through the T5 to T7 segments).

Bartha PRV and the two Bartha mutants used here produce highly specific transneuronal infections in the CNS (8, 9). However, a potential complicating factor could be that PRV caused local, nonspecific infec-

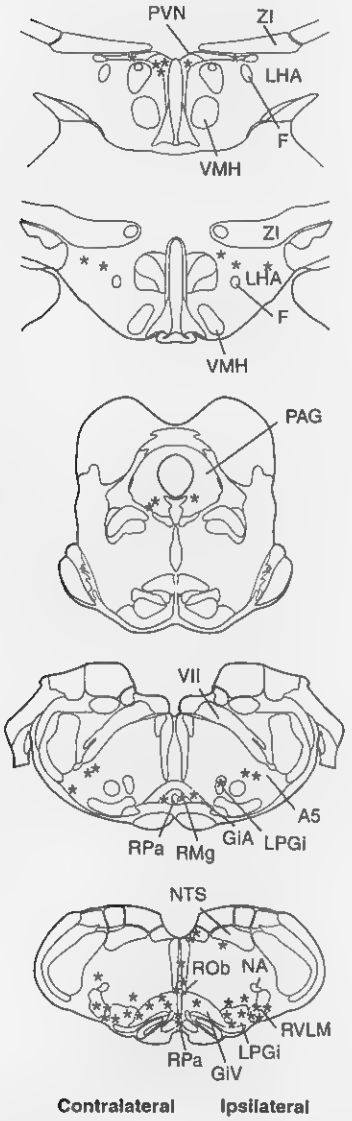

Fig. 2. CNS sites that project to both sympathetic preganglionic outflow systems that innervate stellate ganglion and adrenal gland. Double-labeled neurons are indicated by asterisks. Drawings were made from the computer graphics program Brain Maps (*14*). A5, A5 cell group; F, fornix; GiA, pars alpha region of the gigantocellular reticular nucleus; GiV, ventral region of the gigantocellular reticular nucleus; LHA, lateral hypothalamic area; PVN, paraventricular hypothalamic nucleus; LPGi, lateral paragigantocellular reticular nucleus; NA, nucleus ambiguus; NTS, nucleus tractus solitarius; PAG, periaqueductal gray matter; RMg, raphe magnus nucleus; ROb, raphe obscurus nucleus; RPa, raphe pallidus nucleus; RVLM, rostral ventrolateral medulla; VII, facial nerve; VMH, ventromedial hypothalamic nucleus; ZI, zona incerta.

Table 1. Chemically defined CNS neurons that project to both the stellate and adrenal sympathetic preganglionic outflow systems. In experiment I, Bartha-gC^{Ka} PRV was injected into the stellate ganglion, and Bartha β-galactosidase PRV was injected into the adrenal gland of adult rats (n = 5). The column labeled "identified for chemical" gives the number of double-infected neurons identified for the indicated neuroenzyme or neurotransmitter in a one-in-five series of transverse sections. Data are expressed as mean ± SEM. In experiment II, the data from the converse experiment are presented. Bartha-gC^{Ka} PRV was injected into the adrenal gland, and Bartha β-galactosidase PRV was injected into the stellate ganglion (n = 3).

Brain structure	Number of double-infected neurons			
	Experiment I		Experiment I	
	Total	Identified for chemical	Total	Identified for chemical
		Adrenaline		*Adrenaline*
Rostral ventrolateral medulla	20.5 ± 4.3	11.5 ± 2.9	20.0 ± 7.1	13.7 ± 5.0
Lateral paragigantocellular reticular nucleus	12.0 ± 1.8	8.0 ± 1.5	12.7 ± 2.8	7.3 ± 1.8
Dorsal medulla	5.8 ± 1.9	5.0 ± 2.1	2.0 ± 1.2	1.0 ± 0.6
		Serotonin		*Serotonin*
Raphe obscurus	3.8 ± 1.9	1.4 ± 0.4	3.7 ± 1.9	1.7 ± 0.9
Raphe pallidus	1.8 ± 0.5	0.8 ± 0.2	2.0 ± 0.6	0.0 ± 0.0
Raphe magnus	7.0 ± 2.7	2.6 ± 0.9	8.7 ± 2.2	2.0 ± 0.6
Parapyramidal nucleus	3.0 ± 1.3	1.0 ± 0.3	8.0 ± 3.8	4.0 ± 2.3
		Noradrenaline		*Noradrenaline*
A5 area	14.2 ± 4.4	3.2 ± 1.0	12.3 ± 4.3	2.7 ± 0.9
		Oxytocin		*Oxytocin*
Paraventricular hypothalamic nucleus	8.0 ± 2.6	0.6 ± 0.4	9.7 ± 5.7	0.7 ± 0.7

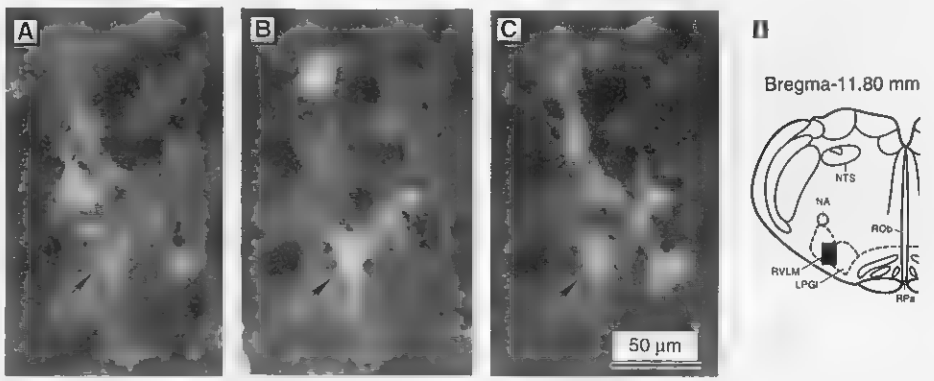

Fig. 3. C1 adrenergic neuron (arrow) that projects to both stellate and sympathoadrenal SPNs. (**A**) gC-positive immunoreactivity in a C1 transneuronally labeled neuron after an injection of Bartha-gC^{Ka} PRV into the stellate ganglion. (**B**) β-Galactosidase immunoreactivity in the same neuron as in (A) after an injection of Bartha β-galactosidase PRV into the adrenal gland. (**C**) Phenylethanolamine-*N*-methyltransferase immunoreactivity in the neuron shown in (A) and (B). (**D**) Line drawing indicating the region (rectangle) illustrated in the photomicrographs. Abbreviations are the same as in Fig. 2. Scale bar = 50 μm.

tions. For example, if PRV spread from one functional class of sympathetic preganglionic neurons (SPNs) to adjacent, functionally unrelated SPNs, this would produce false-positive double labeling in bulbo- and hypothalamospinal projection neurons. To control for this potential problem, we examined each mutant virus for nonspecific lateral spread of viral infection. Stellate SPNs were retrogradely labeled with a standard retrograde neuronal cell body marker, cholera toxin β-subunit, and the ipsilateral sympathoadrenal SPNs were infected with one of the two mutant viruses (*10*). The intermediolateral cell column was examined for double-labeled SPNs in the T5 to T7 spinal regions, which contain maximal overlapping populations of these two different functional classes of neurons. The amount of double labeling served as an index of false-positive labeling. Control experiments with two conventional retrograde neuronal markers established that 4 ± 1 double-labeled SPNs occur in a one-in-two series of longitudinal sections through these three spinal segments (*10*). In the control experiment with Bartha β-galactosidase PRV, 7.9 ± 2.2 (n = 8) SPNs were double labeled, whereas in the control experiment with Bartha gC^{Ka} PRV, 7.5 ± 1.3 SPNs were found (n = 4); neither value was statistically different (two-tailed Student's t test) from the earlier control data (*10*), suggesting that both of the PRV mutants used here produced specific infections in the SPN cell column. In addition, when both viruses were used together, the number of double-labeled SPNs was similar (experiment I, 7.7 ± 2.7 and experiment II, 7.3 ± 0.7). Together with earlier findings that indicate that during a viral infection in the CNS, microglia and macrophages seal off the infected area, enhancing the probability that infections spread in a transsynaptic fashion (*11*), these results indicate that Bartha PRV can be used to produce highly specific infections within functionally defined neural systems.

The two viruses used in this study had genomes similar to the attenuated live vaccine strain (Bartha PRV) that is used in the pig industry to prevent Aujeszky's disease, and each produces specific patterns of transneuronal infections in rats (9). Because Bartha PRV is an attenuated virus, the success rate is relatively low, approaching 20% when used at its optimal titer (6, 7). Therefore, it was not surprising that, when two mutant Bartha variants were used simultaneously, the success rate was 3%. Although this may be regarded as a shortcoming of this method, the qualitative and quantitative results were highly reproducible (Table 1) and were in excellent agreement with earlier single-virus studies (6, 7).

In summary, several CNS regions contain selective subsets of neurons capable of regulating both the cardiac and adrenal sympathetic outflow systems. Although each of these areas has been implicated in autonomic regulation (*12*), it is now clear that there are central neurons potentially capable of producing both sympathetically controlled neural and endocrine (adrenal catecholamine) responses. In all likelihood, these neurons function as command premotor neurons that direct multiple sympathetic responses in a simultaneous and parallel direction. As suggested by Cannon (*13*), they may affect the sympathetic outflow in general like the soft and loud pedals of a piano, by modulating all the notes being played at a given moment.

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4. Bartha gC^{Ka} PRV (100 nl) [(titer = $10^{4.5}$ plaque forming units per milliliter (pfu/ml)] was injected into the right stellate ganglion, and Bartha β-galactosidase PRV (100 nl) (titer = 1×10^7 to 2×10^7 pfu/ml) was injected into the ipsilateral adrenal gland of pentobarbital-anesthetized (50 mg per kilogram of body weight) adult Sprague-Dawley rats of either sex (Sasco, O'Fallon, MO) (175 to 200 g; n = 168); the converse experiment was also performed (n = 88). Out of a total of 256 rats, 30% showed no infection, 62% were infected with only one virus, and 8% exhibited double-virus infections. Within the latter category, 40% (8 rats) displayed highly specific infections for both viruses, whereas the remaining 60% (12 rats) were overinfected and, thus, discarded from our analysis.
5. The rats were reanesthetized with pentobarbital and perfused through the heart with 0.9% saline, followed by 2% paraformaldehyde–25% picric acid made in 0.1 M sodium phosphate buffer (pH 7.0). The brains were removed and processed for immunohistochemistry. Transverse sections of the brain were cut at 50 μm on a freezing microtome and incubated for 16 hours at room temperature in 5% normal donkey serum made in 0.3% Triton-X 100 and 0.02 M potassium phosphate–buffered saline (KPBS) containing three antibodies: goat to β-galactosidase (1:400 dilution) (Arnel Products, New York, NY), mouse monoclonal antibody to gC glycoprotein (ascites fluid) (1:400), and antibodies to one of the following proteins raised in rabbits: phenylethanolamine-*N*-methyltransferase (1:150) (Chemicon, Temecula, CA), tyrosine hydroxylase (1:500) (East-Acres Biologicals, Southbridge, MA), serotonin-bovine serum albumin (1:100) (Incstar, Stillwater, MN), oxytocin (1:500) (Chemicon), or choline acetyltransferase (1:500). The sections were washed twice in KPBS, incubated in 1:100 dilution of biotinylated donkey antibody to mouse immunoglobulin G (IgG) (Jackson ImmunoResearch, West Grove, PA), washed, and then transferred to a solution of tetramethylrhodamine-conjugated streptavidin (1:100) (Jackson), fluorescein isothiocyanate–conjugated donkey antibody to goat IgG (1:50) (Jackson), and 7-amino-4-methylcoumarin-3-acetic acid–conjugated donkey antibody to rabbit IgG (1:50) (Jackson) for 4 hours. The sections were washed, mounted, and a cover slip placed over them with glycerol–phosphate-buffered saline mounting medium containing 0.1% *p*-phenylenediamine to prevent fading.
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Project Aids U.N. Human Rights Efforts

At the request of a top U.N. official, AAAS is designing an information management system that will improve the capability of U.N. human rights treaty monitoring bodies.

The committees are charged with evaluating how well countries are complying with obligations they have agreed to in a number of U.N. human rights covenants and conventions.

"These monitoring procedures have never worked satisfactorily, and the lack of a computerized information management system has contributed to that inadequacy," says Audrey Chapman, director of the AAAS Science and Human Rights Program, which is carrying out the project.

AAAS program associate Stephen Hansen says the system he is developing will make it possible to compare information over the years and across U.N. human rights monitoring bodies, and to integrate data from other sources for more thorough country profiles. "Right now, the treaty monitoring bodies are dependent on what countries tell them," he says. "With this system, committees and staff will be able to have far better information at their disposal when they query states parties, so they may see discrepancies and request additional information." A primary goal, Hansen adds, is to aid the development of early-warning capabilities for evaluating the severity of human rights abuses and the need for emergency intervention.

The mandates of the various human rights covenants are varied, and more than 100 countries are signatories to each agreement, so the information is vast and the monitoring process is complex. But, says Chapman, record-keeping methods at the U.N. Center for Human Rights in Geneva, which serves as the secretariat for all U.N. human rights work, are so outdated that the center "is still consigned to a League of Nations–style paper filing system." Remarkably, "there were no computers until 2 years ago," she notes. Automation has begun, but progress is slow because of scarce resources.

The project was undertaken at the request of Ibrahima Fall, the U.N. assistant secretary-general for human rights. Due for completion next year, it is supported by grants from the United States Institute of Peace and the Joyce Mertz-Gilmore Foundation.

Marking a Milestone

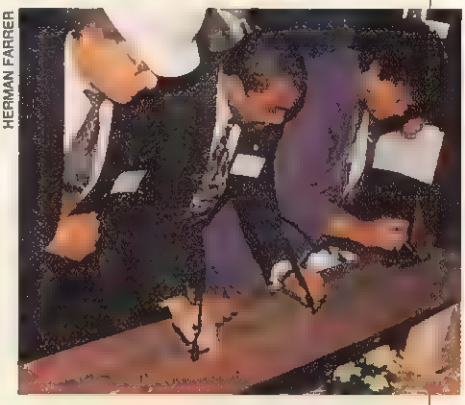
Senior officials of the District of Columbia were among the featured speakers at a "topping out" ceremony last month at the Association's new headquarters in downtown Washington. The event was held to celebrate completion of the first phase of construction of the 12-story building, which will house AAAS and other nonprofit scientific organizations.

Merrick T. Malone (center), D.C. assistant administrator for economic development, and D.C. Council Member Charlene Drew Jarvis (right) signed a symbolic I beam, along with Mike Flynn of Pei Cobb Freed & Partners, the architectural firm.

The project was financed in part by the sale of $52 million in low-interest, tax-exempt revenue bonds authorized by the D.C. government. "I use AAAS often as an example to other nonprofits" of the benefits of the city's revenue bond program, Jarvis said.

AAAS has about 25,000 visitors a year. Association officials estimate that the many specialized facilities in the new Center for Science and Engineering—including a 180-seat auditorium, a model science classroom, conference areas, and a science and technology bookstore—will double that number.

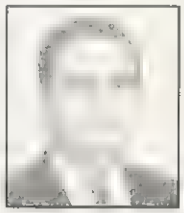
HERMAN FARRER



Caribbean Division Founder Dies

Juan Bonnet-Diez, the founder and first president of the Caribbean Division of AAAS, was killed in Puerto Rico in August when he walked into a convenience store during an armed robbery. He was 56.

Throughout his career, Dr. Bonnet worked to promote the development of science and technology in the Western Hemisphere. He first approached AAAS with the idea of establishing a Caribbean Division. It became a reality in 1984, and as president from 1985-88 he helped shape the direction of the division.



Among its activities, the division last month co-sponsored the Environmental Chemistry Symposium of the Third Pan-American Congress of Chemistry. Despite its being held between Hurricanes Luis and Marilyn, the event drew 1,200 chemists from the Americas and the Caribbean. In December the division will co-sponsor a major conference in San Juan on neuroscience research.

Dr. Bonnet held a Ph.D. in nuclear engineering from the University of Michigan and was the author of more than 100 scientific and technical publications. He had served as director of the University of Puerto Rico Nuclear Center and of the Department of Chemistry and Physics at Bayamon Technical College. In recent years he was director of the graduate program at the Polytechnic University of Puerto Rico, where he also taught.

He had headed several major scientific and engineering organizations, including the Puerto Rico Academy of Sciences and the Pan-American Union of Engineering Associations.

He is survived by a wife and 6 children.

A Decade of Science Education Reform

In 1981, AAAS put science literacy at the top of its priority list and began exploring possibilities for a large-scale project that would bring deep and lasting reform to science education. Next month is the 10th anniversary of the launch of that initiative: Project 2061.

The startup coincided with the 1985 approach of Halley's Comet, prompting the planners to consider all the scientific and technological changes a child entering school in 1985 would witness before the return of the comet in 2061—hence the name.

The project took an ambitious approach, aiming to rebuild K-12 education from scratch. Its report *Science for All Americans* set goals for adult science literacy and has sold more than 100,000 copies since its release in 1989. A companion volume, *Benchmarks for Science Literacy*, was published in 1993 and described what students in grades 2, 5, 8, and 12 should know and be able to do in science, mathematics, and technology.

Project 2061's work has provided the foundation for curriculum reform in many states and school districts and has influenced the development of national science education standards and other reform efforts. The project conducts numerous workshops around the country and continues to develop new reform tools for educators and curriculum developers.

For more information, contact Mary Koppal at 202-326-6643 or by Internet at: mkoppal@aaas.org.

AAAS Members Distinguished for Contributions to Science

In September the AAAS Council elected 273 members as Fellows of AAAS. These individuals will be recognized for their contributions to science at the Fellows Forum to be held on 10 February 1996 during the AAAS Annual Meeting in Baltimore, Maryland. The new Fellows will receive a certificate and a blue and gold rosette pin as a symbol of their distinguished accomplishments. Presented by section affiliations, they are:

Agriculture, Food, and Renewable Resources

Lloyd Lee Anderson, Iowa State Univ. • Robert G. Cassens, Univ. of Wisconsin • Dennis B. Egli, Univ. of Kentucky • William A. Jury, Univ. of California, Riverside • Noel T. Keen, Univ. of California, Riverside • Stephen Kresovich, U.S. Dept. of Agriculture • Rattan Lal, Ohio State Univ. • Terry J. Logan, Ohio State Univ. • Robert H. Miller, Univ. of Rhode Island • Richard J. Norby, Oak Ridge National Lab. • Juan G. Rodriguez, Kentucky Academy of Science • Linda J. Saif, Ohio State Univ. • Michael S. Strauss, AAAS • Goro Uehara, Univ. of Hawaii • Carroll P. Vance, Univ. of Minnesota • Paul H. Williams, Univ. of Wisconsin

Anthropology

Melvin Konner, Emory Univ. • Anna Curtenius Roosevelt, Field Museum of Natural History • Emoke J.E. Szathmary, McMaster Univ. • Kenneth M. Weiss, Pennsylvania State Univ.

Astronomy

Holland C. Ford, Johns Hopkins Univ. • Christine Jones, Harvard-Smithsonian Center for Astrophysics • David W. Latham, Harvard-Smithsonian Center for Astrophysics

Atmospheric and Hydrospheric Sciences

Kenneth H. Brink, Woods Hole Oceanographic Institution • Otis B. Brown, Univ. of Miami • David D. Houghton, Univ. of Wisconsin • James F. Kasting, Pennsylvania State Univ. • Steven C. Wofsy, Harvard Univ.

Biological Sciences

Renato Baserga, Thomas Jefferson Univ. • Harold L. Bergman, Univ. of Wyoming • Alan R. Berkowitz, Institute of Ecosystem Studies • Ralph E. J. Boerner, Ohio State Univ. • Mary Anne Brock, National Institute on Aging • Ann Bucklin, Univ. of New Hampshire • C. John Burk, Smith College • Elizabeth M. Cosper, State Univ. of New York, Stony Brook • Jaleh Daie, Univ. of Wisconsin • Kelvin J. A. Davies, Albany Medical College • Francine Essien, Rutgers Univ. • Theodore Harris Fleming, Univ. of Miami • George Edward Fox, Univ. of Houston • Alice B. Fulton, Univ. of Iowa • Costa Georgopoulos, Univ. of Geneva • Howard Gest, Indiana Univ. • Martha Lee Ulbrick Gillette, Univ. of Illinois • Arturo Gomez-Pompa, Univ. of California, Riverside • Shirley Mae Halling, U.S. Dept. of Agriculture • Steven C. Hand, Univ. of Colorado • G. Miller Jonakait, Rutgers Univ. • Joel E. Keizer, Univ. of California, Davis • Michael R. Landry, Univ. of Hawaii • John Lemons, Univ. of New England • Lars G. Ljungdahl, Univ. of Georgia • Orson K. Miller, Jr., Virginia Polytechnic Institute and State Univ. • Richard L. Nuccitelli, Univ. of California, Davis • Donald Penner, Michigan State Univ. • Nancy N. Rabalais, Louisiana Universities Marine Consortium • Dianna A. Redburn, Univ. of Texas • John McNeill Sieburth, Univ. of Rhode Island • Wayne P. Sousa, Univ. of California, Berkeley • Robert S. Steneck, Univ. of Maine • Donald R. Strong, Bodega Marine Lab. • Cornelius W. Sullivan, National Science Foundation • Stanley A. Temple, Univ. of Wisconsin • Lawrence P. Wackett, Univ. of Minnesota • Patrick J. Walsh, Univ. of Miami • Ellen Cleminshaw Weaver, San Jose State Univ. • George Rickey Welch, Univ. of New Orleans • William J. Wiebe, Univ. of Georgia • Jeffrey F. Williams, Michigan State Univ. • John S. Willis, Univ. of Georgia • Don E. Wilson, Smithsonian Institution • John Edward Wilson, Michigan State Univ. • Karen F. Wishner, Univ. of Rhode Island • Maurice Zeeman, U.S. Environmental Protection Agency

Chemistry

Eugene John Barber, Martin Marietta Energy Systems • Arthur D. Broom, Univ. of Utah • Gary W. Brudvig, Yale Univ. • Hernan Chaimovich, Univ. of Sao Paulo • Burton G. Christensen, Lebanon, N.J. • Helena Li Chum, National Renewable Energy Lab. • F. Fleming Crim, Univ. of Wisconsin • Frans Carl De Schryver, Katholieke Univ. Leuven • Dante Gatteschi, Univ. of Florence • Richard Spencer Givens, Univ. of Kansas • Oskar Max Glemser, Univ. of Gottingen • Frank Jordan, Rutgers Univ. • Irene Emily Kochevar, Harvard Medical School • Edward Kostiner, Univ. of Connecticut • Robert L. Kuczkowski, Univ. of Michigan • Angelo A. Lamola, Rohm and Haas Co. • Robert L. Lichter, Camille and Henry Dreyfus Foundation • Joseph Albert Miller, Jr., DuPont Central Research and Development • Michael D. Morris, Univ. of Michigan • Edmond Murad, Phillips Lab. • Yukito Murakami, Kyushu Univ. • Wilma K. Olson, Rutgers Univ. • Michael C. Pirrung, Duke Univ. • Warren H. Powell, Chemical Abstracts Service • Dallas L. Rabenstein, Univ. of California, Riverside • Kenneth J. Shea, Univ. of California, Irvine • Thomas Wilson Swaddle, Univ. of Calgary • Robert Cooper Taylor, Univ. of Michigan • Klaus H. Theopold, Univ. of Delaware • Michael R. Wasielewski, Argonne National Lab. • W. Henry Weinberg, Univ. of California, Santa Barbara • James K. Whitesell, Univ. of Texas

Dentistry

Malcolm L. Snead, Univ. of Southern California

Education

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Societal Impacts of Science and Engineering
Roger M. Boisjoly, Boisjoly Engineering, Ltd. • Abby Lippman, McGill Univ. • M. Granger Morgan, Carnegie-Mellon Univ. • Joel R. Primack, Univ. of California, Santa Cruz • Michael Traynor, Cooley Godward Castro Huddleson & Tatum, San Francisco

Statistics
George T. Duncan, Carnegie-Mellon Univ. • Iain M. Johnstone, Stanford Univ. • John A. Rice, Univ. of California, Berkeley

BOOK REVIEWS

Invertebrate Immunology

Phylogenetic Perspectives in Immunity. The Insect Host Defense. JULES A. HOFFMANN, CHARLES A. JANEWAY, JR., and SHUNJI NATORI, Eds. Landes, Georgetown, TX, 1994 (distributor, CRC Press, Boca Raton, FL). xviii, 197 pp., illus. $89.95 or £74. Molecular Biology Intelligence Unit.

Like other scientists, immunologists seek basic understandings of complex biological processes; when it comes to complexity, adaptive immunity has few equivalents. An "evolutionary" study provides one means of gathering a simpler or (more realistically) different view of a fundamental form, relationship, or mechanism. However, there are some problems with this approach, not the least of which is that the clues being sought may have long since been erased. Furthermore, the mammalian system, clearly the dominant focus of present biological interest, is so specialized as to be inappropriate for comparison with organisms (such as insects) that pass through major life points in periods that are insignificant relative to the decades that mark the lives of mammals. Ironically, it was studies of invertebrate innate immunity by Elie Mechnikoff in the 1880s that provided the foundation of modern cellular immunology. After more than a hundred years of controversy over the issue of invertebrate immunity, it seems that at least one major group of invertebrate models, the insects, have come into their own. *Phylogenetic Perspectives in Immunity: The Insect Host Defense* summarizes the foundations of our current concepts of the character of immunity in this highly diversified group.

The strength of this work is that its various chapters are focused on critical interpretations of data that largely reflect relevant immune challenges to the species at issue. In case the reader is looking for T and B cells, somatic gene arrangement, and positive selection for increasing affinity of antigen receptors, contributors Boman and Davidson, as well as co-editors Janeway and Hoffmann, clarify the lack of evidence for and improbability of their existence outside of the vertebrates. Along with the excellent introduction to the volume, Boman's opening chapter contains a critical interpretation of his own and others' work, giving the reader a framework for interpreting entomological immunology.

Although little previously unpublished information is put forward in the book, past discoveries, misdirected lines of investigation, and incorrect inferences as well as possible future areas of endeavor are depicted clearly. Several chapters contain summaries of properties of antibacterial or antifungal peptides and other substances and take on an encyclopedic character, but these mercifully avoid farfetched claims of amino acid identities and speculative leaps that postulate homology with the primary mediators of vertebrate adaptive immunity. Even in the encyclopedic approach there is considerable merit—perhaps these molecules afford some new concepts for design of antibiotics, a matter worthy of concern as the dwindling resources of the pharmacologist and chemist are being outpaced by deadly genetic accommodations of infectious agents. The book consistently focuses on immunity in the insect; but you can take what you want from the descriptions of some of the well-established linkages between invertebrate and mammalian immunity—the complement system, mannose-binding proteins, cytokines, and so on. The issues of general similarities between insect immune responses and acute-phase reactants in mammals are not overlooked.

The organization is logical, and as a result of careful ordering of the chapters as well as through that rarest of multi-contributor phenomena, accurate cross-referencing, the book can be read from cover to cover with relative ease. Given these stylistic endorsements, what messages come through? First, the evidence for antibacterial and other responses to infectious agents is compelling. A wide variety of molecules exhibit the effects, and homologs of some can be found in the vertebrates. Certain antibacterial responses are inducible, and Hoffmann *et al.* and Hultmark show that the genes flanking some mediators of antibacterial activity contain transcriptional regulatory sequences that have been implicated in the control of immune processes in mammals. Thus, in the broadest evolutionary context, a thread can be drawn through not only constitutively expressed genes but also the inducible systems.

Perhaps this is not all coincidental. Davidson develops the single most significant evolutionary theme in this text—how major regulatory systems emerge and change. This is not a dissertation on a molecular clock ticking away residue by residue on the face of a structural domain, but rather a consideration of macro events that abruptly alter or integrate developmental and other genetic control processes. Viewed in this light, it is evident that the mediators of insect immunity are unrelated by any stretch of molecular systematics to antibody, but the transcriptional regulation of an immunoglobulin locus and a *Drosophila* inducible response may have much in common. One need not look further than the work emerging recently from the laboratories of Fred Alt and others, in which ancient DNA repair processes lie at the heart of one of the key mechanisms that somatically diversify antibodies and T cell antigen receptors, to comprehend the scope of integration of preexisting systems.

If it is answers that you want as to how the sophisticated mechanisms of segmented gene rearrangement and somatic hypermutation found in all jawed vertebrates evolved, there are several protochordate subphyla and an entire vertebrate class (the Agnatha, or jawless vertebrates) that probably hold more immediately relevant information than can be garnered through studies of insects; however, this book comes as close as any that I have seen to objectively addressing the topic of immunity in a nonvertebrate metazoan and documenting that you do not necessarily need B cell activation and an Fc receptor on a macrophage to kill a bacterium. Given the extraordinary diversity of life form within the insects, it would come as no surprise that there is much more to be learned from these species about the most basic aspects of host response to disease.

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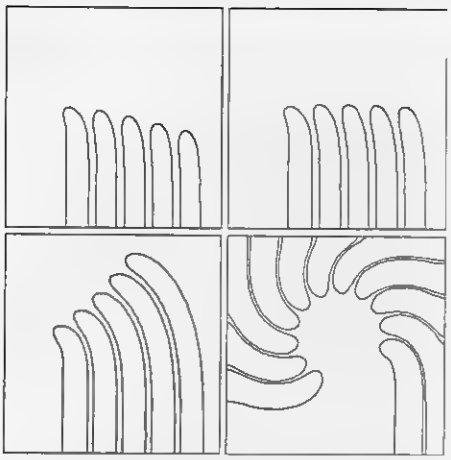
Diffusion Phenomena

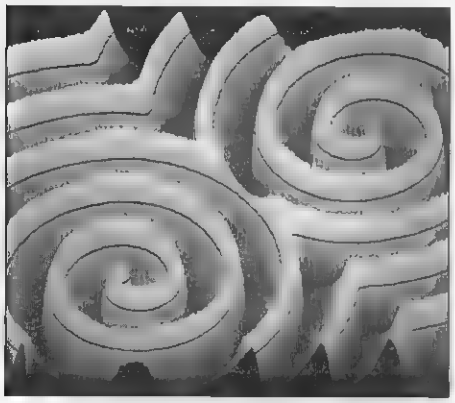
Chemical Waves and Patterns. RAYMOND KAPRAL and KENNETH SHOWALTER, Eds. Kluwer, Norwell, MA, 1995. x, 641 pp., illus. $272 or £179 or Dfl. 425. Understanding Chemical Reactivity, vol. 10.

It might seem that the effect of diffusion in a spatially distributed, unstirred molecular system would be to make homogeneous the spatial concentration distribution of chemical species. However, the opposite can be true. It occurs in complex reacting systems with appropriate feedback loops in their



"Evolution of a broken excitation wave in a reaction-diffusion model of an excitable medium. Contours of constant activator concentration are shown at subsequent time moments for four differed excitabilities of the medium." [From A. S. Mikhailov and V. S. Zykov's paper in *Chemical Waves and Patterns*; V. A. Davidov *et al.* in *Nonlinear Waves in Active Media*, A. Crighton and Yu. Engelbrecht, Eds. (Springer-Verlag, 1989)]

mechanism. Many of these reactions also show temporal oscillation in intermediate concentrations when stirred. Diffusion in such cases can and does lead to the development of spatial concentration gradients supported by free energy dissipation in a far-from-equilibrium state. This fact is of considerable importance in understanding spatial organization in both chemical and biological systems. Thus, in 1906 Robert Luther suggested that coupling of an autocatalytic reaction with diffusion in an excitable medium can lead to traveling waves (now known as trigger waves) of reaction and associated sharp concentration gradients, and that these waves are dynamically



"Symmetric pair of spiral waves in a 1 mm layer of an excitable [Belousov-Zhabotinsky] reaction. The grey levels of transmitted light intensity (490 nm) measured for a 410 × 410 pixel frame are connected by linear interpolation (surface image) and displayed in three-dimensional perspective at a tilt angle of 45°. A narrow iso-intensity interval is enhanced in black." [From S. C. Müller and T. Plesser's paper in *Chemical Waves and Patterns*]

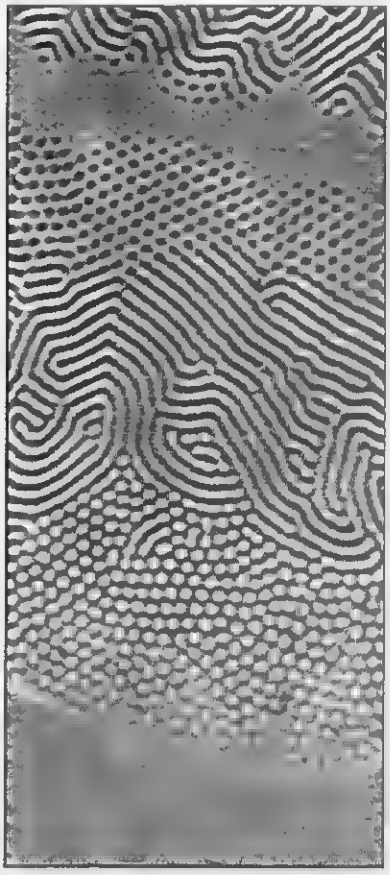
very similar to nerve impulses as well as to other biological information-transfer mechanisms. Though the result was challenged at the time by Walther Nernst, Luther deduced, probably by dimensional analysis, that the velocity of the traveling wave is proportional to the square root of the product of the rate of the autocatalytic reaction and the diffusion coefficient, a result that has proven to be true. When challenged by Nernst on the derivation of this relationship, Luther simply replied, "*aus mir*." It was not proven rigorously until much later, although the connection to nerve impulse transmission was seen by Fisher and Kolmogorov in the 1930s.

Even more remarkably, Alan Turing, the British polymath of enigma-machine fame, demonstrated in 1952 that the coupling of diffusion with reaction can actually destabilize a spatially homogeneous steady state if the chemical mechanism involves both activator and inhibitor species and appropriate feedback loops. The development of stationary concentration patterns then can result from a symmetry-breaking transition occurring as some parameter of the system, such as temperature or a concentration, is varied. This breaking of spatial symmetry results from differences in the diffusion coefficients of the activator and inhibitor. Turing suggested this phenomenon, called a Turing pattern, as a chemical basis of morphogenesis and of pattern formation in general in biological systems.

These profound insights languished until recently for two reasons: there was not a suitable body of theory of nonlinear partial differential equations within which to understand them, and, especially in the case of the Turing patterns, there were no experimentally accessible, chemically well-understood examples. That now has all changed. Experimentally, the Belousov-Zhabotinsky reaction provided, starting in the 1970s, a robust example of trigger waves in a mechanistically well-understood chemical system. Furthermore, in 1990 unstirred flow reactors not subject to convection were cleverly constructed and Turing patterns were discovered under circumstances where their development and properties could be carefully investigated. These advances led to an explosion of experimental and theoretical work and to new insights, all of which this book covers in breadth and depth. Each of the 18 variously authored chapters is well documented to provide a key to the supporting literature.

Trigger-wave dynamics is covered in great detail, including their initiation, the stability of their wave fronts, and their propagation in inhomogeneous media. Disruption of trigger waves either mechanically or in a inhomogeneous medium leads to very complex behaviors. Spiral waves may form in a thin, essentially two-dimensional, layer of reaction medium. Scroll waves of great complexity develop in three dimensions. These structures are expected to be of considerable interest, especially in the area of wave propagation in a muscle such as a beating heart.

The chlorite-iodide-malonic acid (CIMA) system is the only one in which Turing patterns have been seen experimentally. The construction of the reactors is explained, and the phenomena observed so far are systematically reviewed. The chemical mechanism of the CIMA reaction is used to simulate and interpret experimental results. The existence of so many experimental data has prompted significant theoretical



Turing patterns: Stationary concentration patterns resulting from the sole interplay of a local nonlinear chemical reaction (CIMA reaction) and the fickian diffusion of the species in the reacting solution. The sequence of different planforms is obtained in an open spatial reactor with a smooth ramp of control parameter, by E. Dulos, J. Boissonade, and P. De Kepper, contributors to *Chemical Waves and Patterns*; courtesy of P. De Kepper.

investigation into the general properties of reaction-diffusion partial differential equations. In particular the mechanism of pattern formation in terms of the combination of normal spatial modes of perturbations around the spatially homogeneous steady state is reviewed. The connection is made between theory and experiment for various

Vignettes: Concern for Animals

Where the women's movement and the struggle of black women and men have been identity movements, made by those people declared Other by those with power, animal liberation was to be carried out by human beings on behalf of others. Arguably the silence of the oppressed was a significant part of their attraction; the liberators could speak on their behalf unfettered by any voices which said "that is not how I feel, that is not how I am, how dare you presume to speak for me."

—*Hilary Rose*

A question I have often wondered about is whether most students who feel uneasy about dissecting anesthetized animals actually do so out of sympathy with the animals. Would they do whatever is necessary to help an injured animal, or are they just uneasy about handling live animals? I wonder about this especially when I am told that women students are more reluctant to do lab dissections than the men are. Are we really dealing with differences in empathy with other living creatures or with differences in permitted squeamishness?

—*Ruth Hubbard*

—From *Reinventing Biology: Respect for Life and the Creation of Knowledge* (Lynda Birke and Ruth Hubbard, Eds.; Indiana University Press)

bifurcation (qualitative change in behavior) phenomena observed or expected as a control parameter is varied.

A beginning is made at drawing a connection between fluctuations at the microscopic level and macroscopic behavior. Fluctuations may become important near bifurcation points or in chaotic systems, and it seems likely that chemical patterns may be a good area for investigating their effect. These treatments are mainly on the mesoscopic level.

This book provides a current and vital review of an important and active area of research.

Richard J. Field
Department of Chemistry, University of Montana, Missoula, MT 59812, USA

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Books Received

Automation in the Laboratory. W. Jeffrey Hurst, Ed. VCH, New York, 1995. xii, 248 pp., illus. $95.

The Axemaker's Gift. A Double-Edged History of Human Culture. James Burke and Robert Ornstein. Grosset/Putnam, New York, 1995. xviii, 349 pp., illus. $27.95 or $C37.95.

Bacteria. In Biology, Biotechnology and Medicine. Paul Singleton. 3rd ed. Wiley, New York, 1995. x, 319 pp., illus. Paper, $29.95.

Children Solving Problems. Stephanie Thornton. Harvard University Press, Cambridge, MA, 1995. x, 143 pp., illus. $24.95; paper, $10.95. Developing Child.

Climate Change and Agriculture. Analysis of Potential International Impacts. Cynthia Rosenzweig *et al.*, Eds. American Society of Agronomy, Madison, WI, 1995. xviii, 382 pp., illus. Paper, $34. ASA Special Publication no. 59. From a symposium, Minneapolis, Nov. 1992.

Collecting Plant Genetic Diversity. Technical Guidelines. Luigi Guarino, V. Ramanatha Rao, and Robert Reid, Eds. CAB International, Oxford, UK, 1995 (U.S. distributor, University of Arizona Press). xx, 748 pp., illus. $120.

College Physics. Raymond A. Serway and Jerry S. Faughn. 4th ed. Saunders, Philadelphia, 1995. xxvi, 1042 pp., illus., + supplementary material. $73.50.

Color and Light in Nature. David K. Lynch and William Livingston. Cambridge University Press, New York, 1995. xiv, 254 pp., illus. $44.95; paper, $29. 95.

Computer Simulation of Polymers. E. A. Colbourn, Ed. Longman Scientific and Technical, Harlow, Essex, UK, and Wiley, New York, 1994. viii, 343 pp., illus. $185. Polymer Science and Technology.

Confronting Poverty. Prescriptions for Change. Sheldon H. Danziger, Gary D. Sandefur, and Daniel H. Weinberg, Eds. Oxford University Press, New York, 1994. xii, 529 pp., illus. $49.95; paper, $19.95. Based on a conference, Madison, WI, May 1992.

Conserving Wildlife. International Education and Communication Approaches. Susan K. Jacobson, Ed. Columbia University Press, New York, 1995. xxxiv, 302 pp., illus. $45 or £31.50; paper, $22 or £16.75. Methods and Cases in Conservation Science.

Constructing Knowledge in the History of Science. Arnold Thackray, Ed. History of Science Society, Philadelphia, 1995 (distributor, University of Chicago Press). vii, 253 pp., illus. $39; paper, $25; to HSS members, $27.50; paper, $17.50. *Osiris*, 2nd series, vol. 10 (1995).

Cooperative Phenomena in Jahn-Teller Crystals. Michael D. Kaplan and Benjamin G. Vekhter. Plenum, New York, 1995. xvi, 425 pp., illus. $95. Modern Inorganic Chemistry.

Coping with Trouble. How Science Reacts to Political Disturbances of Research Conditions. Uwe Schimank and Andreas Stucke, Eds. Campus, Frankfurt, and St. Martin's, New York, 1994. 401 pp. $59.95.

Coulson and Richardson's Chemical Engineering. Vol. 3, Chemical and Biochemical Reactors and Process Control. J. F. Richardson and D. G. Peacock, Eds. 3rd ed. Pergamon (Elsevier Science), Tarrytown, NY, 1994. xx, 776 pp., illus. $135 or £83; paper, $46 or £28.50.

Critical Issues in Systems Theory and Practice. Keith Ellis *et al.*, Eds. Plenum, New York, 1995. xviii, 712 pp., illus. $79.50. From a conference, Hull, UK, July 1994.

Evolution as Growth of One Earth-Organism. Thomas A. Morrill. Published by the author, Rt. 16, Box 9047, Tallahassee, FL, 1995. vi, 121 pp. Paper, $10 or £10.

Experiment and the Making of Meaning. Human Agency in Scientific Observation and Experiment. David Gooding. Kluwer, Norwell, MA, 1995. xviii, 310 pp., illus. Paper, $29.50 or £19.50 or Dfl. 55. Science and Philosophy, vol. 5. Reprint, 1990 ed.

Social Psychiatry across Cultures. Studies from North America, Asia, Europe, and Africa. Rumi Kato Price, Brent Mack Shea, and Harsha N. Mookherjee, Eds. Plenum, New York, 1995. xvii, 226 pp. $42.50. Topics in Social Psychiatry.

The Solar-Terrestrial Environment. An Introduction to Geospace—the Science of the Terrestrial Upper Atmosphere, Ionosphere and Magnetosphere. J. K. Hargreaves. Cambridge University Press, New York, 1995. xiv, 420 pp., illus. $100; paper, $37.95. Cambridge Atmospheric and Space Science, 5. Reprint, 1992 ed.

Somatic Embryogenesis in Woody Plants. Vol. 1, History, Molecular and Biochemical Aspects, and Applications. S. Mohan Jain, Pramod K. Gupta, and Ronald J. Newton, Eds. Kluwer, Norwell, MA, 1995. xiv, 460 pp., illus. $205 or £134.50 or Dfl. 320. Forestry Sciences, vol. 45.

Space. A Vital Stimulus to Our National Well-Being, and World Space Programs and Fiscal Reality. Gayle L. May *et al.*, Eds. American Astronautical Society, San Diego, 1994 (distributed by Univelt, San Diego). xvi, 318 pp., illus. $70; paper, $50. Science and Technology Series, vol. 83. From symposia, Alexandria and Arlington, VA, April 1992 and March 1993.

The Space Environment. Implications for Spacecraft Design. Alan C. Tribble. Princeton University Press, Princeton, NJ, 1995. xiv, 204 pp., illus. $49.50 or £32.50.

Stability of Superconductors. Lawrence Dresner. Plenum, New York, 1995. xx, 225 pp., illus. $49.50. Selected Topics in Superconductivity.

Stairway to the Mind. The Controversial New Science of Consciousness. Alwyn Scott. Copernicus (Springer-Verlag), New York, 1995. xx, 229 pp., illus. $25 or $C35.

The Stone Skeleton. Structural Engineering of Masonry Architecture. Jacques Heyman. Cambridge University Press, New York, 1995. x, 160 pp., illus. $59.95.

Straight Sex. Rethinking the Politics of Pleasure. Lynne Segal. University of California Press, Berkeley, 1994. xvi, 376 pp. $35; paper, $15. Reprint, 1994 ed.

Structure Correlation. Hans-Beat Bürgi and Jack D. Dunitz, Eds. VCH, New York, 1994. liv, 888 pp., illus. $235.

Sulfate-Reducing Bacteria. Larry L. Barton, Ed. Plenum, New York, 1995. xvi, 336 pp., illus. $85. Biotechnology Handbooks, vol. 8.

Surface Infrared and Raman Spectroscopy. Methods and Applications. W. Suëtaka, with the assistance of John T. Yates, Jr. Plenum, New York, 1995. xiv, 270 pp., illus. $59.50. Methods of Surface Characterization, vol. 3.

Tinkering Toward Utopia. A Century of Public School Reform. David Tyack and Larry Cuban. Harvard University Press, Cambridge, MA, 1995. viii, 184 pp., illus. $22.50.

Two Dimensional Spline Interpolation Algorithms. Helmuth Späth. Peters, Wellesley, MA, 1995. viii, 304 pp., illus. $59.95.

"You Do Teach Atoms, Don't You?" A Case Study in Breaking Science Curriculum Gridlock. Lyman Lyons and Susan Bolyard Millar. LEAD Center, Madison, WI, 1995. xii, 79 pp., illus. Paper, $10.

Publishers' Addresses

Below is information about how to direct orders for books reviewed in this issue. A fuller list of addresses of publishers represented in *Science* appears in the issue of 26 May 1995, page 1220.

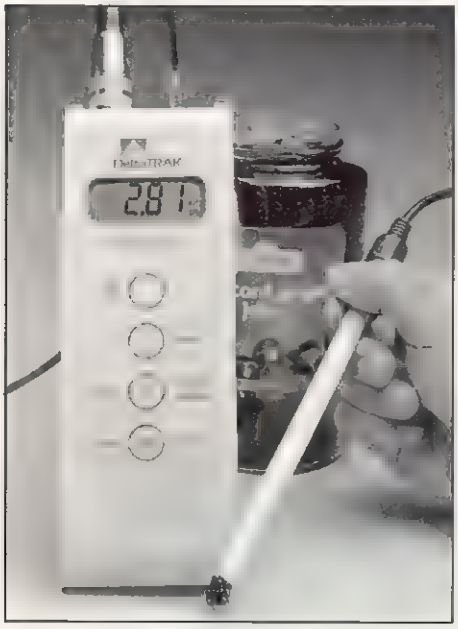
Kluwer Academic Publishers, P.O. Box 358, Accord Sta., Hingham, MA 02018-0358. Phone: 617-871-6600. Fax: 617-871-6528.

R. G. Landes Co., P.O. Box 4858, Austin, TX 78765. Phone: 512-863-7762. Fax: 512-863-0081. E-mail: rglandes@aus.computize.com.

PRODUCTS & MATERIALS

pH Meter

The Model 101 pH Meter is a handheld, portable unit featuring a state of the art ion-sensitive field-effect transistor (ISFET) probe. The ISFET sensor probe is nonglass, rugged, and completely submersible. It stores dry and requires no fill solutions or routine maintenance. The sensor has a 3-s readout with automatic temperature compensation.



It can be used to test clear liquids, semi-solids, dirty samples, and bread dough, and can even measure a single drop of sample. **DeltaTRAK. Circle 138.**

■

Antibodies

Nuclear matrix proteins (NMPs) are becoming recognized for their potential for cancer detection. A range of NMP antibodies includes specificities for prostate cancer (PRO:4-216.1), breast cancer (NM200.4), apoptosis research (204-41), nuclear pore complex (203-37), and spliceosomes (J82, B4A11, 626, B1C8). The antibodies can be used in protein immunoblotting, immunofluorescence, and immunohistochemistry. **Diagnostic BioSystems. Circle 139.**

An antibody that detects a surface glycoprotein found only on a subpopulation of circulating B cells, in mantle cell lymphomas, and in differentiated B cell leukemias is formulated for use in flow cytometry. **Dako Corp. Circle 140.**

■

PCR Clean Up Kit

The PCR Clean Up Kit separates polymerase chain reaction (PCR) products from impurities that interfere with enzymatic labeling or digestion processes. Under specific salt conditions, the kit binds PCR products and separates them from residual PCR components. The bound products can then be eluted from the silica beads by simple low-salt washes. The kit works with a wide size range of products, purifies them quickly, and recovers them efficiently. **Boehringer Mannheim. Circle 141.**

■

Cell Transfection Kit

The Capture-Tec Kit allows the user to create a homogeneous population of transiently transfected cells without creating stable cell lines. The kit features pHook-1 plasmid, which expresses and displays a single-chain antibody to a specific hapten molecule. Users simply cotransfect the pHook-1 plasmid with their own constructs, then isolate transfected cells with hapten-coated magnetic beads to study posttransfection intracellular events. Selected cells can be used for differential display, protein immunoblots, flow cytometry, enzymatic analysis, and pulse-chase experiments. **Invitrogen. Circle 142.**

■

Genomic DNA Isolation Kit

The DNA Multiprep kit provides a novel procedure for the simultaneous processing of multiple genomic DNA samples from whole blood, mammalian cells, and select tissues. The procedure is neither labor-intensive nor time-consuming, nor does it involve the use of hazardous organic solvents. The entire

Newly offered instrumentation, apparatus, and laboratory materials of interest to researchers in all disciplines in academic, industrial, and government organizations are featured in this space. Emphasis is given to purpose, chief characteristics, and availability of products and materials. Endorsement by *Science* or AAAS is not implied. Additional information may be obtained from the manufacturers or suppliers named by circling the appropriate number on the Readers' Service Card and placing it in a mailbox. Postage is free.

procedure is performed in one 96-well plate. It entails adding three reagents to the samples with a multichannel pipette. The DNA bound to the plate surface can be used directly for restriction enzyme digest, Southern blot, or polymerase chain reaction. The kit consistently gives high molecular weight DNA preparations of high yield and purity. The DNA prepared is suitable for large-scale screening, forensic testing, medical diagnostics, and reverse genetics. **Biotecx Laboratories. Circle 143.**

■

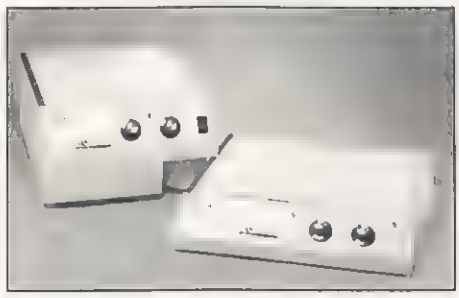
Graphics and Data Analysis Software

Origin 4.0 is a new version of a Windows-based technical graphics and data analysis program. The redesign includes new analysis and graphics tools, a user-friendly and more powerful nonlinear curve fitter, and a toolbar-based user interface for instant access to analysis tools and common features. The new two-dimensional graphics toolbar allows users to instantly access many graph types, including vector and polar graphs as well as line, scatter, area, bar, pie, and statistical charts. New features include baseline and peak analysis for spectroscopy, improved fast Fourier transform that facilitates digital signal processing, and a powerful nonlinear curve fitter with approximately 200 built-in functions. The program is compatible with Windows 95 and requires only a 386/DX or higher processor, 4 MB random access memory, Windows 3.1 or later, and 4 MB hard drive space. **Microcal Software. Circle 144.**

■

Gel Dryers

Model GD 40/50 is a slab gel dryer with a large 40 cm by 50 cm surface capable of quickly drying standard size sequencing gels as well as multiple smaller gels. The design



features a vacuum seal provided by a transparent silicone screen and a porous, polypropylene sheet. The unit has an adjustable heater permitting settings from 30° to 80°C and a timer that runs up to 5 hours adjusted in 5-min intervals. The Model GDS 2000 Gel Dryer combines the attributes of the GD 40/50 with a vacuum pump and chemical trap. **Life Technologies. Circle 145.**

Literature

496 MOS Multiple Organic Synthesizer is a brochure on an instrument that can perform a variety of solid phase organic syntheses. The unit offers reaction heating and cooling, environmentally controlled reactions, multispeed vortex mixing, and more. **Advanced ChemTech. Circle 146.**

Reagents for Cytoskeletal and Neuromuscular Research highlights a line of these products. **Alexis Biochemicals. Circle 147.**

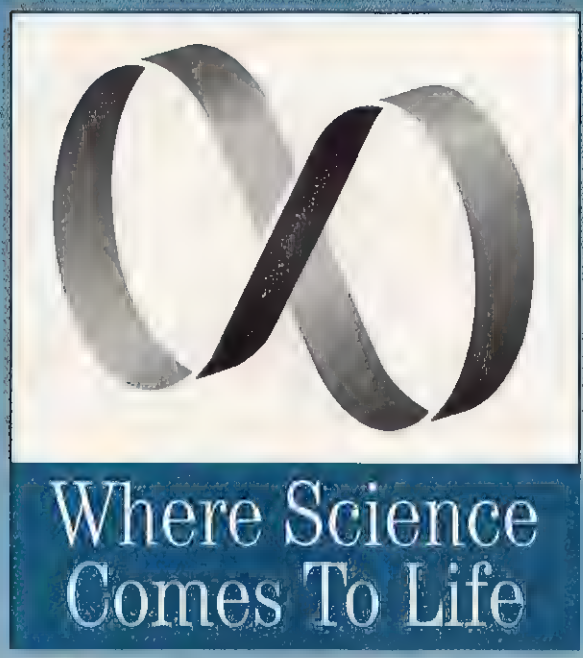
A series of application notes covers many facets of rapid kinetics measurements and illustrates an enormous range of applications for the manufacturer's line of equipment. **Hi-Tech Scientific. Circle 148.**

Fluorescent Gel Mobility Shift Assay is an application note on an assay used to study DNA protein binding in the *Escherichia coli* DNA mismatch repair system. Fluorescent gel shift assays can also be used to determine the affinity, abundance, binding constants, and binding specificity of DNA binding proteins. **Vistra Systems. Circle 149.**

Revco Laboratory CO_2 Incubators highlights products designed for growing viruses and cells without desiccation over a broad range of temperatures, humidities, and CO_2 control levels. **Revco Scientific. Circle 150.**

AMSIE '96

AAAS Annual Meeting & Science Innovation Exposition



LEARN FROM THE EXPERTS

Discover recent developments in risk assessment, environmental clean up, and sustainability

Explore the latest in medical research: AIDS, the human genome, neuroscience, and cardiovascular science

Find out about new issues in government science policy and funding

Get full details on science education reform

AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

Inside

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Where Science Comes To Life

AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

October, 1995

Dear Colleague:

The **AAAS Annual Meeting and Science Innovation Exposition** is the preeminent multidisciplinary event for scientists. It is my pleasure to invite you to attend AMSIE'96 and to join an array of scientists, educators, and policymakers in examining issues and emerging research from all areas of science.

This year's meeting offers exciting new programming that promises to affirm our interdisciplinary tradition. Researchers from every facet of science – medical, environmental, evolutionary, physical, social and technological – will convene to exchange and publicize new knowledge. In fact, more than 5,000 of your colleagues are expected to be in Baltimore to share their latest research advances, explore the fertile synergies between disciplines, and debate today's critical issues in science policy and education.

The theme for AMSIE'96 – **Where Science Comes to Life** – sets the tone for a unique blend of symposia, topical and plenary lectures, specialized seminars, and poster presentations. Some special highlights:

This year's **Science Innovation** offers exceptional symposia covering progress in neuroscience, cardiovascular science, AIDS, and genome research. Topical lectures will highlight emerging developments in instrumentation and research methodology ... see page 13 of this program.

The Origins Seminar features leading scientists presenting their most recent ideas, research and information on how the universe and the world around us have evolved. Presentations will range from researching the Big Bang and stellar origins to present understanding of biological evolution ... detailed information on page 12.

Other AMSIE'96 highlights include: presentations by senior policymakers on the future of science and technology research funding and a seminar focused on helping scientists master the Internet ... see pages 10 and 12 for more details.

Also of particular interest – field trips to important and educational science centers in the Baltimore area; career development seminars; over 100 exhibits of publishers, computer companies, government agencies, and scientific equipment companies; and Employment Exchange services, which are free to AAAS members.

AMSIE'96 offers numerous opportunities to meet, network and learn from many of the leading figures in various fields of science. Please join us in Baltimore, the city where, indeed, science comes to life. Register now, and take advantage of the advance registration discount rates (see forms on page 16 and 17). I look forward to seeing you at AMSIE'96.

Sincerely,

Rita R. Colwell

Rita Colwell
AAAS President and AMSIE'96 Program Chair

AMSIE '96

Time Table

FEBRUARY 8-13, 1996
BALTIMORE
CONVENTION CENTER
BALTIMORE, MARYLAND

THURSDAY, 8th	FRIDAY, 9th	SATURDAY, 10th	SUNDAY, 11th	MONDAY, 12th	TUESDAY, 13th
Seminar Registration 7.00 AM - Noon Stouffer Renaissance	**Meeting Registration** 7:00 AM - 5:00 PM Convention Center	**Meeting Registration** 7.00 AM - 5:00 PM Convention Center	**Meeting Registration** 7:00 AM - 5:00 PM Convention Center	**Meeting Registration** 7.00 AM - 5.00 PM Convention Center	**Meeting Registration** 8.00 AM - 1:00 PM Convention Center
	Concurrent Sessions 8:30 AM - 11:30 AM Convention Center Hyatt Regency Stouffer Renaissance See program for details.	**Concurrent Sessions** 8.30 AM - 11:30 AM Convention Center Hyatt Regency Stouffer Renaissance See program for details.	**Concurrent Sessions** 8:30 AM - 11:30 AM Convention Center Hyatt Regency Stouffer Renaissance See program for details.	**Concurrent Sessions** 8 30 AM - 11.30 AM Convention Center Hyatt Regency Stouffer Renaissance See program for details.	**Concurrent Sessions** 8:30 AM - 11:30 AM Convention Center Hyatt Regency See program for details.
	Science Innovation Sessions 8:30 AM - 11:30 AM Convention Center	**Science Innovation** Sessions 8:30 AM - 11:30 AM Convention Center	**Science Innovation** Sessions 8.30 AM - 11:30 AM Convention Center	**Science Innovation** Sessions 8:30 AM - 11:30 AM Convention Center	**Science Innovation** Sessions 8:30 AM - 11:30 AM Convention Center
Seminar: Origins **Seminar: Internet** 8:30 AM - 11:30 AM Stouffer Renaissance	**Seminar: Origins** **Seminar: Internet** 8:30 AM - 11:30 AM Stouffer Renaissance				**Field Trip** Columbus Science Ctr NASA 8:30 AM - 12:30 PM
Public Science Day 9 00 AM - 2:00 PM Convention Center	**AJAS Oral** Presentations 8:30 AM - 11:30 AM Convention Center	**Exhibition** 10 00 AM - 3:00 PM Convention Center	**Exhibition** 10:00 AM - 3.00 PM Convention Center	**Exhibition** 10:00 AM - 2:00 PM Convention Center	**DelMarVa** Poster Sessions 10:30 AM - 2:30 PM
	Student Awards Poster Competition 10:00 AM - 2:00 PM Convention Center	**EE Career Seminars** 8:30 AM - 5:30 PM Convention Center	**EE Career Seminars** 8:30 AM - 5:30 PM Convention Center	**EE Career Seminars** 8:30 AM - 5:30 PM **EE Job Fair** 10.00 AM - 4:00 PM Convention Center	**EE Job Fair** 10:00 AM - 4:00 PM Convention Center
Field Trips Columbus Science Ctr Johns Hopkins Lab 1:00 PM - 5:00 PM		**Physical/Life Sciences** Poster Sessions 10:30 PM - 2:30 PM Convention Center	**Social Sci/Education** Poster Sessions 10:30 PM - 2:30 PM Convention Center		**Field Trip** U.S. Dept of Agriculture 12:30 PM - 5:00 PM
Meeting Registration 2:00 PM - 7:00 PM Convention Center	**Topical Lectures** 1:00 PM - 2:00 PM SI'96 Topical Lectures 8:30 AM - 9:15 AM 1:30 PM - 2:15 PM See program for details	**Topical Lectures** 1:00 PM - 2:00 PM SI'96 Topical Lectures 8:30 AM - 9:15 AM 1:30 PM - 2:15 PM See program for details	**Topical Lectures** 1:00 PM - 2:00 PM SI'96 Topical Lectures 8:30 AM - 9:15 AM 1:30 PM - 2:15 PM See program for details	**Topical Lectures** 1:00 PM - 2:00 PM SI'96 Topical Lectures 8:30 AM - 9:15 AM 1:30 PM - 2:15 PM See program for details	**Topical Lectures** 1:00 PM - 2:00 PM SI'96 Topical Lectures 8:30 AM - 9:15 AM 1:30 PM - 2:15 PM See program for details
Seminar: Origins **Seminar: Internet** 2:30 PM - 5:30 PM Stouffer Renaissance	**Seminar: Origins** **Seminar: Internet** 2:30 PM - 5:30 PM Stouffer Renaissance	**Awards Ceremony** Noon - 1:00 PM Hyatt Regency	**Science Innovation** Poster Sessions 10:30 AM - 2:30 PM Convention Center		
	Concurrent Sessions 2:30 PM - 5:30 PM Convention Center Hyatt Regency Stouffer Renaissance See program for details.	**Concurrent Sessions** 2:30 PM - 5:30 PM Convention Center Hyatt Regency Stouffer Renaissance See program for details.	**Concurrent Sessions** 2:30 PM - 5:30 PM Convention Center Hyatt Regency Stouffer Renaissance See program for details.	**Concurrent Sessions** 2.30 PM - 5:30 PM Convention Center Hyatt Regency Stouffer Renaissance See program for details.	**Concurrent Sessions** 2:30 PM - 5:30 PM Convention Center Hyatt Regency See program for details.
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	Exhibit Grand Opening 4:00 PM - 6.30 PM Reception 5:00 PM - 6:30 PM Convention Center				
	AJAS Poster Session 4.00 PM - 6:30 PM Convention Center				
Opening Session: Allocating Research Funds 6:00 PM - 8:00 PM Hyatt Regency	**Plenary Lecture** Neal Lane 6:30 PM - 7:30 PM Convention Center	**President's Lecture** Rita R. Colwell 6:30 PM - 7:30 PM Hyatt Regency	**Plenary Lecture** Jacqueline Barton 6:30 PM - 7:30 PM Hyatt Regency	**Plenary Lecture** David Satcher 6:30 PM - 7:30 PM Hyatt Regency	
		President's Reception 7:30 PM - 8:30 PM Hyatt Regency			

MEETING-AT-A-GLANCE

Time Schedule
All AM sessions: 8:30 AM - 11:30 AM (unless otherwise indicated)
All PM sessions: 2:30 PM - 5:30 PM (unless otherwise indicated)

TRACK	FRIDAY, 9TH	SATURDAY, 10TH	SUNDAY, 11TH	MONDAY, 12TH	TUESDAY, 13TH
Assessing and Managing Risk	**AM** *Prudent Practices in Laboratory Handling and Disposal of Chemicals **PM** *Safety and Pollution Prevention: Maximizing Research Innovation, Minimizing Regulatory Burden	**AM** Innovative Chemical Separations and the Clean-up of National Nuclear Sites **PM** Siting Hazardous Facilities: Views on Fair Process and Outcome	**AM** Risk Assessment: The New Panacea Regulatory Morass or Just a Tool? **PM** Science of Decision Making in a Complex World	**AM** Social Learning in the Management of Global Environmental Risks **PM** Assessing Health and Environmental Risks from Long-term Radiation Contamination in Chelyabinsk, Russia	**AM** *Biosafety: Issues of Risk and Responsibility in Biotechnology
Biological Diversity	**AM** The Science of Biodiversity	**PM** The Ethics of Human Genetic Enhancement	**AM** Cultural Diversity in Psychological Structure and Functioning **AM** Building Evolutionary Trees: Evidence and Analysis **AM/PM** Coral Reefs: Global Change and Biodiversity Loss **PM** Diversity of Human Physiological Adaptability	**AM/PM** Heterochrony: Merging Evolutionary Perspectives in Paleoanthropology, Paleontology, and Biology **AM/PM** Utilizing Africa's Genetic Affluence through Natural Products, Research, and Development	**AM** *The Microbial World: Foundation of the Biosphere **AM** Ethnogeriatrics: Biodiversity of Disease and Death Among Elderly People of Color **PM** *Microbial Diversity: The Unseen Treasure
Educating for the Future	**AM** Assessing Progress: Lessons Learned from Some Informal Science Education Programs **AM** Quantitative Literacy and Science Education **PM** Comprehensive School Health Programs: Promoting Public Health Through K-12 Education **PM** The National Science Foundation and Urban Science Education	**AM** From Curiousity to Science Through Linguistic Inquiry **AM** Scientists and Urban Science Education **PM** Reforming Elementary Science Education in Urban Districts **PM** The Textbook of the Future	**AM** Assessing Systemic Reform from National Perspectives to Student Outcomes **AM** EFARR Education: Giving Meaning to the Sciences of Life **PM** K-12 Math and Science Education is Change Really Taking Place? **PM** What Works: Successful University-based Outreach Programs	**AM** The Federal Role in Education Effects of Changing Attitudes **AM/PM** Pseudoscience: Biology and the Education of African American Studies **PM** 2:30 PM - 4:00 PM Using Problem-based Learning to Bolster Achievement for all Students **PM** 4:00 PM - 5:30 PM Problem-based Learning in Undergraduate Science	**AM** "School Crossings" on the Information Superhighway **PM** Building Environmental Science Curricula for Youth and Undergraduates
Environment and Science on the Chesapeake	**AM** The Middle Atlantic Environmental and Ecological Systems: Strategies for Sustainable Development		**PM** Preservation of Farmland and Open Space in the Northeast	**AM** Our Lancaster County (PA) Neighbors: The Old Order Amish **AM** Science, Citizens, and Public Policy in the Chesapeake Bay Program **PM** *Biotechnology in Maryland **PM** The Restoration of the Chesapeake: A National Paradigm	
Environment and Sustainability	**AM** How Many People Can the Earth Support? **PM** Consumption and Population Growth: Twin Challenges to Sustainable Development	**AM** International Perspectives on Population, Consumption, and the Environment: Science and Policy Issues **PM** Limits to Agricultural Productivity **PM** Recovering Tropical Forests: Conservation, Development, and Local Communities	**AM/PM** The U.S. Environmental and Natural Resource Research Strategy	**AM** Dredged Material Disposal and Waste Management in the Nearshore Environment **PM** *Biotechnology and Aquaculture: Finfish Reproduction, Growth, and Development	**AM** *Engineering Functionality into Foods: Benefits for the Consumer
Examining Global Change	**AM** The Open Exchange of Environmental Information	**AM/PM** Climate Change: The Second Assessment Report of the Intergovernmental Panel on Climate Change	**AM** Reconfiguration of Floodplain Management Since the Mid-continent Floods **AM** Remote Sensing of Climate Sensitive Parameters from Space **PM** Social Scientists Contributions to Remote Sensing	**AM** Assessing Ecological Implications of Changes in Climate **PM** Stratospheric Ozone Depletion by Halogens: Recent Achievements, and Continuing Challenges	**AM** Surface Age Dating in Environmental Studies and Reconstruction of Global Change **PM** Interactions of Karst Geology and Ecology
Industry, Technology, and Engineering	**PM** Ocean Science and Engineering Two Cultures Meet in the Ocean	**AM** Science and Technology in the NAFTA Framework **PM** High-Performance Work Transformations in Advanced Manufacturing	**PM** Industry/DOE Laboratory Technology Partnerships: Do the Taxpayers Benefits	**AM/PM** *Stimulating the Local Economy With High Tech Jobs: A Status Report	**AM/PM** New Directions in Industrial Research and Development
Information Age	**AM** *Frontier Applications in Computer Methods for Dynamic Systems	**AM** *Artificial Life in Cyberspace **PM** National Information Infrastructure: Access and Use at the People and Policy Level	**AM** Building the Health Information Highway: Prognosis for a Cure **PM** Health Research and Education Information for the Next Century	**AM** *Intelligent Agents: Software Assistants in Finding, Retrieving, and Analyzing Information	**AM** Cyberscience: Scientific Life Evolving on the Internet
Mathematics and the Physical Sciences	**AM/PM** Human Exploration of Space **PM** Recent Trends in Japanese Science and Technology	**AM/PM** *Frontiers of the Physical Sciences, 1996 **AM/PM** *All Eyes on the Universe: Multi-wavelength Astrophysics **PM** The Universe: Views from Space	**AM** New Studies of Meteorites **AM** 9:30 AM - 11:30 AM *Mathematics for the Next Century: Knots **PM** 2:15 PM - 4:15 PM *Mathematics for the Next Century: Pattern Formation **PM** 4:15 PM - 6:15 PM *Mathematics for the Next Century: Geometric Arrangements with Applications to Physics and Robotics **PM** The Discovery of Radioactivity: The Birth of Nuclear Physics **PM** The Search for Extraterrestrial Intelligence	**AM** *New Directions in Atomic, Molecular, and Optical Science: Controlling Matter with Light **AM** Impact of the Global Positioning System (GPS) **PM** *New Physics in the Baltimore/Washington Area	

Time Schedule
All AM sessions: 8:30 AM - 11:30 AM (unless otherwise indicated)
All PM sessions: 2:30 PM - 5:30 PM (unless otherwise indicated)

TRACK	FRIDAY, 9TH	SATURDAY, 10TH	SUNDAY, 11TH	MONDAY, 12TH	TUESDAY, 13TH
Neuroscience, Behavior, and Language	**AM** The Architecture of the Sign in Signed Languages **AM** *The Developing Brain: Genes, Environment, and Behavior **PM** *The Brains Behind Human Communication: New Approaches to Research	**AM** *New Approaches to the Neurobiology of Language **PM** Sexual Differences in Brain and Behavior	**AM** Deterioration or Evolution? Language Standards and Linguistics **AM/PM** Neural Network and Symbolic Approaches to Language	**AM** The Mind's Clocks: Circadian and Interval Timing	**AM** Limbic System Our Mammalian Heritage: Normal/Deviating Emotion, Memory, Homicidal Action
Policy, Science, and Engineering	**AM** Young Scientists in Transition: Career Challenges and Science Policy Implications **PM** "Science Studies" and the Advancement of Science **PM** *Science and the Editorial Page	**AM** Assessing Competitiveness in Research **AM** Implementation Strategies for "Platform for Action": The U.N. Conference on Women	**AM** Science, Technology, and the States **PM** *Impacts of the 104th Congress on Science and Technology	**AM** Societal Returns to Investment in Research: New Directions, New Concerns **PM** *Communicating with Policymakers: Strategies for Scientists and Engineers **PM** Immigration of Scientists and Engineers: Social and Economic Impacts	**AM** *Science Careers in a Changing Economy **AM** The Critical Appraisal of Science and Technology Policy Analysis **PM** Innovative Collaboration Student-Faculty Partnerships Explore Curriculum Development
Practice of Science	**AM** Analytic Approaches to Fairness **PM** Advances in Peer Review Research	**AM/PM** Not Just Mentors: Educating for the Responsible Conduct of Research	**AM** Research Ethics: We're All Accountable, So Who's Accounting **PM** Ethical Standards for Science and Engineering		**AM** Science Survival: A Practicum
Public Health and Medicine	**AM** Entertaining, Educational or Alarming? Public Health Issues and Popular Perceptions **AM** *Medicines Discovery in the 21st Century **PM** Health Care Reform and the Future of the Academic Medical Center **PM** *Mathematical Problems in the Diagnosis and Treatment of Disease	**AM** *Scope of the AIDS Epidemic in the United States **PM** *AIDS: Epidemiology, Biology, and Prevention of HIV Infection **PM** *Molecular Medicine Enters the Mouth	**AM** 1995: A Year of Dramatic Shifts in Health Data Policy **AM/PM** *Replacement Parts and Implantations for People **AM** Religious, Social, and Environment Factors that Influence Disease States **PM** Life Long Health: Beyond the Medical Model	**AM/PM** *Global Change and Emerging Infectious Diseases	**AM/PM** *Challenges in the Development of Medications for Opiate and Cocaine Addictions **PM** Alzheimer's Disease: Prospects for the Future
Science and International Security		**PM** Ballistic Missile Defense	**AM** International Arms Control: Lessons from the UNSCOM Experience	**AM** A Comprehensive Test Ban Treaty: Implications for National Security and Non-Proliferation **PM** An Assessment of the Nunn-Lugar Cooperative Threat Reduction Program	**AM** Environmental Stress and Violence in the Developing World **PM** Environmental Refugees and Ecological Restoration
Science and Society	**AM** Intersection of Environment, Health, Professional Ethics, and Law **AM** The Sense of Justice: Evolutionary Origins of Moral and Legal Behavior **PM** Science, Technology, and Disability: 20th Anniversary Reflections **PM** Women Nobelists: Their Work Their Lives, and Their Impact on Science	**AM** Growing Up American: Dilemmas of the New Second Generation **AM/PM** Uncertainty, Science, and the Media **PM** Women and Violence: Victims, Offenders, and Prevention Policies	**AM** Major Mental Disorder and Crime: New Data, New Policies? **PM** Histories and Philosophies **PM** Are Women Succeeding In Science?	**AM** The General Public's View about Science and Reasoning **PM** Life After 65: How Science Can Promote Successful Aging	**AM/PM** Whither Research-Intensive Universities?
Science for All	**AM/PM** Science for the Naked Eye, XXIII **PM** Making the Past Alive through Ceramics: Physical Sciences Meet Anthropology	**AM** 9:30 AM - 11:30 AM Youth Meets the Masters **PM** Science is Fun!	**AM** Contributions of Science to Human Rights Truth Commissions	**PM** Mysteries of the American Landscape	**AM** Science Museums: Past, Present, and Future
SCIENCE INNOVATION	**AM** DNA Computing **AM/PM** Neuroscience and Technology: Non-Invasive Functional Brain Mapping **AM/PM** Signal Transduction **PM** Quantum Computation	**AM** Neuroscience and Technology: Visual Memory **AM** Structural and Molecular Basis of Cancer Vaccines **PM** Neuroscience and Technology: Unique Experimental Protocols **PM** Chromosomal Instability as a Causal Factor in Cancer **PM** Structural Biology	**AM** Genomics: Bacteria and Plants **AM** Cardiovascular Science and and Technology: Gene Therapy for Vascular Disease **AM/PM** Advances in AIDS Research **PM** Cardiovascular Science and Technology: Vascular Biology: Viruses as a Cause of Cardiovascular Disease **PM** Genomics: Animals	**AM** Cardiovascular Science and Technology: Cardiac MRI **AM** Reproductive and Developmental Biology: Innovations in Fertility **AM/PM** Advances in AIDS Research **PM** Cardiovascular Science and Technology: Diseases of the Cytoskeleton and Contractile Apparatus **PM** Reproductive and Developmental Biology: Developmental Biology	

SEMINARS
Origins: Thursday, 16th and Friday, 17th, AM/PM
Living with the Internet: Thursday, 16th and Friday, 17th, AM/PM

Note: All information as of 9/1/95. Any changes, additions,or deletions received after that date will be included in the next printing of this program.

(*) = Indicates sessions that may also be of interest to Science Innovation attendees.

PLENARY AND TOPICAL

Lectures

FEBRUARY 8-13, 1996
BALTIMORE
CONVENTION CENTER
BALTIMORE, MARYLAND

All information is as of 9/1/95. Changes, additions or deletions received after that date will be included in the next printing of this program.

Indicates sessions that may also be of interest to Science Innovation attendees

PLENARY LECTURES

THURSDAY, FEBRUARY 8, 1996

OPENING CEREMONIES AND LECTURE

6:00pm-8:00pm

A panel discussion of the National Academy of Science (NAS) report on research funding featuring Dr. Frank Press*, Carnegie Institution of Washington and former President, NAS; and senior administration and congressional representatives*.

FRIDAY, FEBRUARY 9, 1996

KEYNOTE LECTURE

6:30pm-7:30pm

Neal Lane, National Science Foundation

SATURDAY, FEBRUARY 10,1996

PRESIDENT'S LECTURE

6:30pm-7:30pm

Rita R. Colwell, President, AAAS and President, University of Maryland-Biotechnology Institute

SUNDAY, FEBRUARY 11,1996

PLENARY LECTURE

Jacqueline Barton, Chemistry Department, California Institute of Technology

MONDAY, FEBRUARY 12, 1996

PLENARY LECTURE

David Satcher*, Director, U.S. Centers for Disease Control and Prevention

TOPICAL LECTURERS

(Tentative List)

FRIDAY, FEBRUARY 9, 1996

1:00pm-2:00pm

Daniel S. Goldin, National Aeronautics and Space Administration

Alvin M. Liberman, Haskins Laboratories

Jocelyn Elders*, Arkansas Children's Hospital

FRIDAY, FEBRUARY 9, 1996

SARTON LECTURE:

1:00pm-2:00pm

Jane Maienschein, Arizona State University

SATURDAY, FEBRUARY 10, 1996

1:00pm-2:00pm

France Cordova, National Aeronautics and Space Administration and Pennsylvania State University

Teresa Fryberger*, U.S. Department of Energy

Robert T. Watson*, Office of Science and Technology Policy

SUNDAY, FEBRUARY 11, 1996

1:00pm-2:00pm

D. James Baker, National Oceanic and Atmospheric Administration

John H. Conway*, Princeton University

MCGOVERN LECTURE IN THE BEHAVIORAL SCIENCES:

SUNDAY, FEBRUARY 11, 1996

1:00PM-2:00PM

James L. McGaugh, University of California, Irvine

MONDAY, FEBRUARY 12, 1996

1:00PM-2:00PM

Alison Brooks*, George Washington University

Yelena Yesha, University of Maryland

TUESDAY, FEBRUARY 13, 1996

1:00PM-2:00PM

Kristin Shrader Frechette, University of South Florida

Alan Leshner, National Institute on Drug Abuse

Wylie Poag, U.S. Geological Survey

SCIENCE INNOVATION TOPICAL LECTURES

AM = 8:30AM-9:15AM

PM = 1:30PM-2:15PM

FRIDAY AM

Watt W. Webb*, Cornell University

SATURDAY AM

Flossie Wong-Staal, University of California-San Diego

SATURDAY PM

Susanne Ildstad, University of Pittsburgh

SUNDAY AM

Robert Bonner, National Institutes of Health

SUNDAY PM

William A. Haseltine, Human Genome Sciences

MONDAY AM

William Bialek, NEC Research Institute

MONDAY PM

Steven M. Block, Princeton University

(*) = invited, not yet confirmed

HOTEL FORM – SEE PAGE 17

Assessing and Managing Risk

AM = 8:30AM-11:30AM

PM = 2:30PM-5:30PM

Prudent Practices in Laboratory Handling and Disposal of Chemicals

FRIDAY AM

Org by Edward M. Arnett, Duke Univ, and Tamae Wong, Natl Resch Council

Safety and Pollution Prevention: Maximizing Research Innovation, Minimizing Regulatory Burden

FRIDAY PM

Org by Edward M. Arnett, Duke Univ

Innovative Chemical Separations and the Clean-up of National Nuclear Sites

SATURDAY AM

Org by William L. Kuhn, Pacific Northwest Lab

Siting Hazardous Facilities: Views on Fair Process and Outcome

SATURDAY PM

Org by Joanne Linnerooth-Bayer, Intl Inst for Applied Systems Analysis

Risk Assessment: The New Panacea Regulatory Morass or Just a Tool?

SUNDAY AM

Org by William F. Isherwood, Lawrence Livermore Natl Lab, and Don Ritter, Natl Environmental Policy Inst

Science of Decision Making in a Complex World

SUNDAY PM

Org by Alice F. Healy, Univ of Colorado, and Francis A. Beer, Univ of Colorado

Social Learning in the Management of Global Environmental Risks

MONDAY AM

Org by Miranda A. Schreurs, Univ of Maryland, and William C. Clark, Harvard Univ

Assessing Health and Environmental Risks from Long-term Radiation Contamination in Chelyabinsk, Russia

MONDAY PM

Org by Elizabeth J. Kirk, AAAS

Biosafety: Issues of Risk and Responsibility in Biotechnology

TUESDAY AM

Org by Dianne Janczewski, U.S. Agency for Intl Development, Vanderlei Perez Canhos, Fundacao Andre Tosello, and Jeff Starn, AAAS

Biological Diversity

AM = 8:30AM-11:30AM

PM = 2:30PM-5:30PM

The Science of Biodiversity

FRIDAY AM

Org by Joan Abrahamson, Jefferson Inst, Peter Crane, Field Museum, and Anna Roosevelt, Field Museum

The Ethics of Human Genetic Enhancement

SATURDAY PM

Org by William Gardner, Univ of Pittsburgh, and Erik Parens, The Hastings Ctr

Coral Reefs: Global Change and Biodiversity Loss

SUNDAY AM, PM

Org by Gene Rosenberg, Smithsonian Institution

Cultural Diversity in Psychological Structure and Functioning

SUNDAY AM

Org by Hazel Rose Markus, Stanford Univ

Building Evolutionary Trees: Evidence and Analysis

SUNDAY AM

Org by Martin Farach, Rutgers Univ

Diversity of Human Physiological Adaptability

SUNDAY PM

Org by Peter W. Hochachka, Univ of British Columbia

Heterochrony: Merging Evolutionary Perspectives in Paleoanthropology, Paleontology, and Biology

MONDAY AM, PM

Org by Nancy Minugh-Purvis, Med College of Pennsylvania, and Michael L. McKinney, Univ of Tennessee

Utilizing Africa's Genetic Affluence through Natural Products, Research, and Development

MONDAY AM, PM

Org by Amy Auerbacher Gimbel, AAAS, and Berhanu Abegaz, Univ of Botswana

The Microbial World: Foundation of the Biosphere

TUESDAY AM

Org by Rita R. Colwell, Univ of Maryland, and James T. Staley, Univ of Washington

Ethnogeriatrics: Biodiversity of Disease and Death Among Elderly People of Color

TUESDAY AM

Org by Haragopal Thadepalli, Charles R. Drew Univ of Med and Sci

Microbial Diversity: The Unseen Treasure

TUESDAY PM

Org by Sivramiah Shantharam, U.S. Dept of Agriculture, and L. Val Giddings, U.S. Dept of Agriculture

Educating for the Future

AM = 8:30AM - 11:30AM

PM = 2:30PM - 5:30PM

Assessing Progress: Lessons Learned From Some Informal Science Education Programs

FRIDAY AM

Org by Marilee Long, Colorado State Univ, and Jocelyn Steinke, Western Michigan Univ

Quantitative Literacy and Science Education

FRIDAY AM

Org by Cathy Crocker, Amer Statistical Assoc

The National Science Foundation and Urban Science Education

FRIDAY PM

Org by James R. Oglesby, AAAS, and Mary Koppal, AAAS

Comprehensive School Health Programs: Promoting Public Health Through K-12 Education

FRIDAY AM

Org by Valerie Setlow, Inst of Med

From Curiosity to Science Through Linguistic Inquiry

SATURDAY AM

Org by Maya Honda, Wheelock College, and Wayne O'Neil, Massachusetts Inst of Tech

Scientists and Urban Science Education

SATURDAY AM

Org by Jo Ellen Roseman, AAAS, and Mary Koppal, AAAS

REGISTRATION FORM – SEE PAGE 16

Reforming Elementary Science Education in Urban Districts

SATURDAY PM

Org by Leon Ukens, Towson State Univ

The Textbook of the Future

SATURDAY PM

Org by Caroline M. Eastman, Univ of South Carolina

Assessing Systemic Reform from National Perspectives to Student Outcomes

SUNDAY AM

Org by Jane Butler Kahle, Miami Univ

EFARR Education: Giving Meaning to the Sciences of Life

SUNDAY AM

Org by Vernon B. Cardwell, Univ of Minnesota, and James Ellis, Bio Sci Curriculum Study

K-12 Math and Science Education: Is Change Really Taking Place?

SUNDAY PM

Org by George Campbell, Natl Action Council for Minorities in Engineering

What Works: Successful University-based Outreach Programs

SUNDAY PM

Org by Bonnie Schmidt, Univ of Western Ontario

The Federal Role in Education: Effects of Changing Attitudes

MONDAY AM

Org by George Campbell, Natl Action Council for Minorities in Engineering

Pseudoscience, Biology and the Education of African American Studies

MONDAY AM, PM

Org by Joseph L. Graves, Arizona State Univ - West, and Bernard Ortiz de Montellano, Wayne State Univ

Using Problem Based Learning to Bolster Achievement for All Students

MONDAY 2:30PM-4:00PM

Org by Maxine E. Bleich, Ventures in Education

Problem-Based Learning in Undergraduate Science

MONDAY 4:00PM-5:30PM

Org by Harold B. White, Univ of Delaware, and Barbara Duch, Univ of Delaware

"School Crossings" on the Information Superhighway

TUESDAY AM

Org by Rosanne W. Fortner, Ohio State Univ

Building Environmental Science Curricula for Youth and Undergraduates

TUESDAY PM

Org by Shobha Sriharan, Virginia State Univ, Neil Grigg, Colorado State Univ, and Florence Dunkel, Montana State Univ

Environment and Science on the Chesapeake

AM = 8:30AM - 11:30AM

PM = 2:30PM - 5:30PM

The Middle Atlantic Environmental and Ecological Systems: Strategies for Sustainable Development

FRIDAY AM

Org by Christopher K. Mooers, Univ of Miami, and Frederick Grassle, Rutgers Univ

Preservation of Farmland and Open Space in the Northeast

SUNDAY PM

Org by Dale Colyer, West Virginia Univ

Our Lancaster County (PA) Neighbors: The Old Order Amish

MONDAY AM

Org by Victor A. McKusick, Johns Hopkins Schl of Med

Science, Citizens, and Public Policy in the Chesapeake Bay Program

MONDAY AM

Org by M. Grant Gross, Chesapeake Resch Consortium

Biotechnology in Maryland

MONDAY PM

Org by William A. Haseltine, Human Genome Sciences

The Restoration of the Chesapeake: A National Paradigm

MONDAY PM

Org by Ray Miller, Univ of Maryland, and Alan W. Taylor, Univ of Maryland

Environment and Sustainability

AM = 8:30AM - 11:30AM

PM = 2:30PM - 5:30PM

How Many People Can the Earth Support?

FRIDAY AM

Org by Joel E. Cohen, Rockefeller Univ

Consumption and Population Growth: Twin Challenges to Sustainable Development

FRIDAY PM

Org by A.R. Palmer, Inst for Cambrian Studies, Albert A. Bartlett, Univ of Colorado, and E-An Zen, Univ of Maryland

International Perspectives on Population, Consumption, and the Environment: Science and Policy Issues

SATURDAY AM

Org by Victoria Dompka, AAAS

Limits to Agricultural Productivity

SATURDAY PM

Org by Shu Geng, Univ of California-Davis, and Donald A. Holt, Univ of Illinois

Recovering Tropical Forests: Conservation, Development, and Local Communities

SATURDAY PM

Org by David Barton Bray, Inter-American Foundation

The U.S. Environmental and Natural Resource Research Strategy

SUNDAY AM, PM

Org by D. James Baker, Natl Oceanic and Atmospheric Admin, and Ronald Pulliam, Natl Biol Serv

Dredged Material Disposal and Waste Management in the Nearshore Environment

MONDAY AM

Org by Gerald M. Friedman, Brooklyn College

Biotechnology and Aquaculture: Finfish Reproduction, Growth, and Development

MONDAY PM

Org by Yonathan Zohar, Ctr of Marine Biotech, and Jack Greer, Maryland Sea Grant College

Engineering Functionality into Foods: Benefits for the Consumer

TUESDAY AM

Org by Gerald E. Gaull, Georgetown Ctr for Food and Nutrition Policy, and Robin Yeaton Woo, Georgetown Ctr for Food and Nutrition Policy

Examining Global Change

AM = 8:30AM - 11:30AM

PM = 2:30PM - 5:30PM

The Open Exchange of Environmental Information

FRIDAY PM

Org by Roberta Balstad Miller, Consortium of Intl Earth Science Infor Networks, and Peter M. Banks, Environmental Resch Inst of Michigan

HOTEL FORM – SEE PAGE 17

Climate Change: The Second Assessment Report of the Intergovernmental Panel on Climate Change

SATURDAY AM, PM

Org by Robert Watson, Office of Sci and Tech Policy, and Richard Moss, U.S. Global Change Resch Prog

Reconfiguration of Floodplain Management Since the Midcontinent Floods

SUNDAY AM

Org by John F. Shroder, Univ of Nebraska-Omaha

Remote Sensing of Climate Sensitive Parameters from Space

SUNDAY AM

Org by Kristina B. Katsaros, Univ of Washington

Social Scientists Contributions to Remote Sensing

SUNDAY PM

Org by Emilio F. Moran, Indiana Univ

Assessing Ecological Implications of Changes in Climate

MONDAY AM

Org by Mary Barber, Sustainable Biosphere Office, and Jeremy Eddy, Sustainable Biosphere Office

Stratospheric Ozone Depletion by Halogens: Recent Achievements, and Continuing Challenges

MONDAY PM

Org by Manvendra K. Dubey, SRI Intl, and Darin W. Toohey, Univ of California-Irvine

Surface Age Dating in Environmental Studies and Reconstruction of Global Change

TUESDAY AM

Org by John F. Shroder, Univ of Nebraska-Omaha, and M. Povitch, U.S. Geol Survey

Interactions of Karst Geology and Ecology

TUESDAY PM

Org by Daniel L. Chess, IBM Corp, and David Luckins, Natl Speleological Soc

Industry, Technology, and Engineering

AM = 8:30AM - 11:30AM

PM = 2:30PM - 5:30PM

Ocean Science and Engineering: Two Cultures Meet in the Ocean

FRIDAY PM

Org by Karl S. Pister, Univ of California, and Peter Brewer, Monterey Bay Aquarium Resch Inst

Science and Technology in the NAFTA Framework

SATURDAY AM

Org by Miguel Jose Yacaman, Univ Natl Autonoma de Mexico, and G. Ahmed Meer, U.S. Embassy, Mexico

High-Performance Work Transformations in Advanced Manufacturing

SATURDAY PM

Org by Joel Yudken, Work and Tech Inst

Industry/DOE Laboratory Technology Partnerships: Do the Taxpayers Benefit?

SUNDAY PM

Org by Tina M. Kaarsberg, Vista Technologies, Inc, and Robert Olson, Inst for Alternative Futures

Stimulating the Local Economy with High Tech Jobs: A Status Report

MONDAY AM, PM

Org by Barry G. Silverman, George Washington Univ, Sally Rood, Natl Tech Transfer Ctr, and Christopher T. Hill, George Mason Univ

New Directions in Industrial Research and Development

TUESDAY AM, PM

Org by Christopher T. Hill, George Mason Univ, and Daniel Berg, Rensselaer Polytech Inst

Information Age

AM = 8:30AM-11:30AM

PM = 2:30PM-5:30PM

Frontier Applications in Computer Methods for Dynamic Systems

FRIDAY AM

Org by Ronald E. Mickens, Clark Atlanta Univ

Artificial Life in Cyberspace

SATURDAY AM

Org by Maureen C. Kelly, BIOSIS, and Caroline M. Eastman, Univ of South Carolina

National Information Infrastructure: Access and Use at the People and Policy Level

SATURDAY PM

Org by Norine Noonan, Florida Inst of Tech, and Melinda Bier, Florida Inst of Tech

Building the Health Information Highway: Prognosis for a Cure

SUNDAY AM

Org by Barry G. Silverman, George Washington Univ, and Bettijoyce B. Lide, Natl Inst of Standards and Tech

Health Research and Education: Information for the Next Century

SUNDAY PM

Org by Marion J. Ball, Univ of Maryland, and Elliot Siegel, Natl Library of Med

Intelligent Agents: Software Assistants in Finding, Retrieving, and Analyzing Information

MONDAY PM

Org by Bonnie C. Carroll, Infor Intl Assoc

Cyberscience: Scientific Life Evolving on the Internet

TUESDAY AM

Org by Alexander Fowler, AAAS

Mathematics and the Physical Sciences

AM = 8:30AM - 11:30AM

PM = 2:30PM - 5:30PM

Human Exploration of Space

FRIDAY AM, PM

Org by Yoji Kondo, NASA-Goddard Space Flight Ctr

Recent Trends in Japanese Science and Technology

FRIDAY PM

Org by Masa-Toshi Koshiba, Japan Soc for the Promo of Sci, and Richard Getzinger, AAAS

Frontiers of the Physical Sciences, 1996

SATURDAY AM, PM

Org by Rolf M. Sinclair, Natl Sci Foundation

All Eyes on the Universe: Multiwavelength Astrophysics

SATURDAY AM, PM

Org by Saeqa Vrtilek, Univ of Maryland

The Universe - Views from Space

SATURDAY PM

Org by Ron Allen, Johns Hopkins Univ

New Studies of Meteorites

SUNDAY AM

Org by Rolf M. Sinclair, Natl Sci Foundation

Mathematics for the Next Century: Knots

SUNDAY 9:30AM-11:30AM

Org by Cameron Gordon, Univ of Texas-Austin, and Charles Radin, Univ of Texas-Austin

Mathematics for the Next Century: Pattern Formation

SUNDAY 2:15PM-4:15PM

Org by Martin Golubitsky, Univ of Houston

REGISTRATION FORM – SEE PAGE 16

Mathematics for the Next Century: Geometric Arrangements with Applications to Physics and Robotics

SUNDAY 4:15PM-6:15PM

Org by Janos Pach, New York Univ, and Charles Radin, Univ of Texas-Austin

The Discovery of Radioactivity: The Birth of Nuclear Physics

SUNDAY PM

Org by Saul Krasner, U.S. Coast Guard Academy

The Search for Extraterrestrial Intelligence

SUNDAY PM

Org by Lori Marino, Emory Univ, and Donald Tarter

New Directions in Atomic, Molecular, and Optical Science: Controlling Matter with Light

MONDAY AM

Org by John Weiner, Univ of Maryland

Impact of the Global Positioning System (GPS)

MONDAY AM

Org by P. Kenneth Seidelmann, U.S. Naval Observatory, and William Klepczynski, U.S. Naval Observatory

New Physics in the Baltimore/Washington Area

MONDAY PM

Org by Robert L. Park, Amer Physical Soc

Neuroscience, Behavior, and Language

AM = 8:30AM-11:30AM

PM = 2:30PM-5:30PM

The Architecture of the Sign in Signed Languages

FRIDAY AM

Org by Richard P. Meier, Univ of Texas-Austin

The Developing Brain: Genes, Environment, and Behavior

FRIDAY AM

Org by Christine Hohmann, Morgan State Univ

The Brains Behind Human Communication: New Approaches to Research

FRIDAY PM

Org by James F. Kavanagh, Amer Speech-Language-Hearing Assoc, and Judith L. Lauter, Univ of Oklahoma-Health Sci Ctr

New Approaches to the Neurobiology of Language

SATURDAY AM

Org by David Caplan, Massachusetts Gen Hosp

Sexual Differences in Brain and Behavior

SATURDAY PM

Org by Arthur P. Arnold, Univ of California

Deterioration or Evolution?: Language Standards and Linguistics

SUNDAY AM

Org by Geoffrey Nunberg, Xerox PARC

Neural Network and Symbolic Approaches to Language

SUNDAY AM, PM

Org by Paul Smolensky, Johns Hopkins Univ

The Mind's Clocks: Circadian and Interval Timing

MONDAY AM

Org by John Gibbon, Columbia Univ, and Rae Silver, Barnard College

Limbic System, Our Mammalian Heritage: Normal/Deviating Emotion, Memory, Homicidal Action

TUESDAY AM

Org by Anneliese A. Pontius, Massachusetts Gen Hosp

Policy, Science, and Engineering

AM = 8:30AM - 11:30AM

PM = 2:30PM - 5:30PM

Young Scientists in Transition: Career Challenges and Science Policy Implications

FRIDAY AM

Org by Eliene Augenbraun, U.S. Agency for Intl Development

"Science Studies" and The Advancement of Science

FRIDAY PM

Org by A. Edward Manier, Univ of Notre Dame

Science on the Editorial Page

FRIDAY PM

Org by Rick E. Borchelt, Exec Offc of the President, and Lynne Friedmann, Friedmann Communication

Assessing Competitiveness in Research

SATURDAY AM

Org by Albert H. Teich, AAAS

Implementation Strategies for "Platform for Action": The U.N. Conference on Women

SATURDAY AM

Org by Dominique Homberger, Louisiana State Univ, Catherine J. Didion, Assoc for Women in Sci, and Linda Thompson, AAAS

Science, Technology, and the States

SUNDAY AM

Org by David H. Guston, Rutgers Univ

Impacts of the 104th Congress on Science and Technology

SUNDAY PM

Org by Steve Nelson, AAAS and Dana Isherwood, Lawrence Livermore Natl Lab

Societal Returns to Investment in Research: New Directions, New Concerns

MONDAY AM

Org by Kenneth M. Brown, Natl Sci Foundation

Communicating with Policymakers: Strategies for Scientists and Engineers

MONDAY PM

Org by Steve Nelson, AAAS

Immigration of Scientists and Engineers: Social and Economic Impacts

MONDAY PM

Org by Sharon G. Levin, Univ of Missouri

Science Careers in a Changing Economy

TUESDAY AM

Org by Alan Fechter, Committee on Pros in Sci and Tech, and Paula M. Rayman, Radcliffe Pub Policy Inst

The Critical Appraisal of Science and Technology Policy Analysis

TUESDAY AM

Org by David H. Guston, Rutgers Univ

Innovative Collaboration: Student-Faculty Partnerships Explore Curriculum Development

TUESDAY PM

Org by Susan Higman, Student Pugwash USA

Practice of Science

AM = 8:30AM-11:30AM

PM = 2:30PM-5:30PM

Analytic Approaches to Fairness

FRIDAY AM

Org by Steven J. Brams, New York Univ

Advances in Peer Review Research

FRIDAY PM

Org by Art Stamps, Inst of Environmental Quality

Not Just Mentors: Educating for the Responsible Conduct of Research

SATURDAY AM, PM

Org by Diane Hoffman-Kim, Massachusetts Inst of Tech, and Stephanie J. Bird, Massachusetts Inst of Tech

HOTEL FORM – SEE PAGE 17

Research Ethics: We're All Accountable, So Who's Accounting?

SUNDAY AM

Org by C.K. Gunsalus, Univ of Illinois, and Drummond Rennie, Inst for Health Policy Studies

Ethical Standards for Science and Engineering

SUNDAY PM

Org by Mark S. Frankel, AAAS

Science Survival: A Practicum

TUESDAY AM

Org by Susan Higman, Student Pugwash USA

Public Health and Medicine

AM = 8:30AM-11:30AM

PM = 2:30PM-5:30PM

Entertaining, Educational, or Alarming? Public Health Issues and Popular Perceptions

FRIDAY AM

Org by Marcel LaFollette, Ctr for Intl Sci, and Tech Policy, Sharon Dunwoody, Univ of Wisconsin, and James C. Cornell, Harvard-Smithsonian Ctr for Astrophysics

Medicines Discovery in the 21st Century

FRIDAY AM

Org by David J. Triggle, State Univ of New York-Buffalo

Health Care Reform and the Future of the Academic Medical Center

FRIDAY PM

Org by Barry M. Brenner, Harvard Med Schl

Mathematical Problems in the Diagnosis and Treatment of Disease

FRIDAY PM

Org by David Isaacson, Rensselaer Polytec Inst

Scope of the AIDS Epidemic in the United States

SATURDAY AM

Org by Philip S. Rosenberg, Natl Cancer Inst

AIDS: Epidemiology, Biology, and Prevention of HIV Infection

SATURDAY PM

Org by Richard T. Johnson, Johns Hopkins Hosp, and Thomas C. Quinn, Johns Hopkins Univ

Molecular Medicine Enters the Mouth

SATURDAY PM

Org by Lawrence A. Tabak, Univ of Rochester

1995: A Year of Dramatic Shifts in Health Data Policy

SUNDAY AM

Org by John S. Gardenier, Ctrs for Disease Control, and R. Clifton Bailey, Health Care Financing Admin

Replacement Parts and Implantations for People

SUNDAY AM, PM

Org by Robert S. Langer, Massachusetts Inst of Tech, and James G. Martin, Carolinas Med Ctr

Religious, Social, and Environmental Factors That Influence Disease States

SUNDAY AM

Org by Harold G. Koenig, Duke Univ-Med Ctr, and David B. Larson, Natl Inst for Healthcare Resch

Life Long Health: Beyond the Medical Model

SUNDAY PM

Org by Robert M. Schmidt, California Pacific Med Ctr

Global Change and Emerging Infectious Diseases

MONDAY AM, PM

Org by Paul R. Epstein, Harvard Schl of Pub Health, and Rita R. Colwell, Univ of Maryland

Challenges in the Development of Medications for Opiate and Cocaine Addictions

TUESDAY AM, PM

Org by Constance Pechura, Natl Academy of Sci, Charles V. Grudzinskas, Natl Inst on Drug Abuse, and Carolyn Fulco, Inst of Med-Natl Academy of Sci

Alzheimer's Disease: Prospects for the Future

TUESDAY PM

Org by Claudia H. Kawas, Johns Hopkins Univ-Schl of Med

Science and International Security

AM = 8:30AM - 11:30AM

PM = 2:30PM - 5:30PM

Ballistic Missile Defense

SATURDAY PM

Org by Scott D. Sagan, Ctr for Intl Security and Arms Control

International Arms Control: Lessons from the UNSCOM Experience

SUNDAY AM, PM

Org by Raymond A. Zilinskas, Univ of Maryland-Biotech Inst, and Ed Lacey, U.S. Arms Control, and Disarmament Agency

A Comprehensive Test Ban Treaty: Implications for National Security and Nonproliferation

MONDAY AM

Org by Gregory van der Vink, IRIS Consortium, and Thomas Wander, AAAS

An Assessment of the Nunn-Lugar Cooperative Threat Reduction Program

MONDAY PM

Org by William Potter, Monterey Inst for Intl Studies

Environmental Stress and Violence in the Developing World

TUESDAY AM

Org by Thomas Homer-Dixon, Univ of Toronto

Environmental Refugees and Ecological Restoration

TUESDAY PM

Stuart M. Leiderman, Univ of New Hampshire

Science and Society

AM = 8:30AM-11:30AM

PM = 2:30PM-5:30PM

Intersection of Environment, Health, Professional Ethics, and Law

FRIDAY AM

Org by Colin L. Soskolne, Univ of Alberta, and Laura Westra, Univ of Windsor

The Sense of Justice: Evolutionary Origins of Moral and Legal Behavior

FRIDAY AM

Org by Roger D. Masters, Dartmouth College, and Margaret Gruter, Gruter Inst for Law and Behavioral Resch

Science, Technology, and Disability: 20th Anniversary Reflections

FRIDAY PM

Org by Virginia W. Stern, AAAS

Women Nobelists: Their Work, Their Lives, and Their Impact on Science

FRIDAY PM

Org by Jaleh Daie, Univ of Wisconsin, and Catherine J. Didion, Assoc for Women in Sci

Growing Up American: Dilemmas of the New Second Generation

SATURDAY AM

Org by Ruben G. Rumbaut, Michigan State Univ

Uncertainty, Science, and the Media

SATURDAY AM, PM

Org by Carol L. Rogers, Sharon Dunwoody, Univ of Wisconsin, and Sharon M. Friedman, Lehigh Univ

REGISTRATION FORM – SEE PAGE 16

Women and Violence: Victims, Offenders, and Prevention Policies

SATURDAY PM

Org by Roland Chilton, Univ of Massachusetts

Major Mental Disorder and Crime: New Data, New Policies?

SUNDAY AM

Org by Sheilagh Hodgins, Univ de Montreal

Histories and Philosophies

SUNDAY PM

Org by A. Edward Manier, Univ of Notre Dame

Are Women Succeeding in Science?

SUNDAY PM

Org by Mary Frank Fox, Georgia Inst of Tech

The General Public's View About Science and Reasoning

MONDAY AM

Org by Susan Losh, Florida State Univ

Life After 65: How Science Can Promote Successful Aging

MONDAY PM

Org by Phyllis Moen, Cornell Univ

Whither Research - Intensive Universities?

TUESDAY AM, PM

Org by C. Judson King, Univ of California, and Irwin Feller, Pennsylvania State Univ

Science for All

AM = 8:30AM-11:30AM

PM = 2:30PM-5:30PM

Science for the Naked Eye, XXIII

FRIDAY AM, PM

Org by Rolf M. Sinclair, Natl Sci Foundation

Making the Past Alive through Ceramics: Physical Sciences Meet Anthropology

FRIDAY PM

Org by Charles C. Kolb, Natl Endowment for Humanities, and Dean E. Arnold, Wheaton College

Youth Meets the Masters

SATURDAY 9:30AM-11:30AM

Org by Saul Krasner, U.S. Coast Guard Academy (Ret.)

Science is Fun!

SATURDAY PM

Org by Bassam Z. Shakhashiri, Univ of Wisconsin

Contributions of Sceince to Human Rights Truth Commissions

SUNDAY AM

Ory by Daniel Salcedo, AAAS

Mysteries of the American Landscape

MONDAY PM

Org by Walter Sullivan, The New York Times

Science Museums: Past, Present, and Future

TUESDAY AM, PM

Org by Marc Rothenberg, Smithsonian Institution, and Michele Aldrich, AAAS

AMSIE '96

Seminars

Origins

Org by Stephen P. Maran, NASA-Goddard Space Flight Ctr and Joel S. Levine, NASA-Langley Resch Ctr.

This will be an examination of the latest research into the subject of origins. The first day will be devoted to origins of the universe and its elements. It will include the most current ideas, hypotheses, theories, and information from the Hubble Space Telescope and other instruments. The second day will focus on origins of our solar system, the earth, its atmosphere, and on the prebiotic and biotic origins of life.

THURSDAY, FEBRUARY 8,

The Big Bang and the Origin of the Universe
John C. Mather, NASA-Goddard Space Flight Ctr

Large Scale Structure in the Universe and its Origin
Mario Livio, Space Telescope Inst

The Evolution of Galaxies: Protogalaxies to the Milky Way
Anne L. Kinney, Space Telescope Inst

The Orgins of Time
Paul Davies, University of Adelaide

The Birth of Stars
Alan P. Boss, Carnegie Institution of Washington

The Origin of Comets
Anita L. Cochran, Univ of Texas-Austin

Stellar Evolution: From Protostars to Supernovae and Black Holes
Richard C. Henry, Johns Hopkins Univ

FRIDAY, FEBRUARY 9

The Origin of the Solar System
George W. Wetherill, Carnegie Institution of Washington

The Origin of the Earth
David J. Stevenson, California Inst of Tech

The Origin of the Atmosphere
Joel S. Levine, NASA-Langley Resch Ctr

The Origin of Life: Prebiotic Organic Synthesis,
Sherwood Chang, NASA-Ames Resch Ctr

Early Life: The Geological Record
J. William Schopf, Univ of California-Los Angeles

Late Life: Biological Evolution
Lynn Margulis, Univ of Massachusetts-Amherst

Living with the Internet

Org by John W. Berry, Univ of Illinois-Chicago, Alice Calabrese, Chicago Library System, and Robert F. Palank, St. Louis Comm College

THURSDAY, FEBRUARY 8
FRIDAY, FEBRUARY 9

What is the Internet? How do I get to it? Once there, what can I do? What software do I need? Simply put, this seminar will equip you to successfully work on the information superhighway. The 1 1/2 day workshop will cover the basics of internet operation, electronic mail, telenet, file transfer, world wide web, netscape, mosaic, gopher, and much more.

In addition, plans are being developed to have hands-on workshops available so that you can try out your new skills. These workshops are presently planned for Saturday, February 10 and Sunday, February 11 in a special facility near the Exhibit Hall. Seminar registrants will be afforded a special opportunity during the seminar to register for these sessions.

HOTEL FORM – SEE PAGE 17

Science Innovation presents advances in neuroscience, cardiovascular science, AIDS, and genome research. Afternoon topical lectures will highlight emerging developments in instrumentation and research methodology. *(See topicals on page 6.)*

AM = 8:30AM - 11:30AM

PM = 2:30PM - 5:30PM

Neuroscience and Technology: Non-Invasive Functional Brain Mapping

FRIDAY AM, PM

Org by Marcus Raichle, Washington Univ-Schl of Med

Neuroscience and Technology: Visual Memory

SATURDAY AM

Org by Robert Desimone, Natl Insts of Health

Neuroscience and Technology: Unique Experimental Protocols

SATURDAY PM

Org to be announced

Cardiovascular Science and Technology: Gene Therapy for Vascular Disease

SUNDAY PM

Org to be announced

Cardiovascular Science and Technology: Vascular Biology: Viruses as a Cause of Cardiovascular Disease

SUNDAY AM

Org by Stephen Epstein, NIH

Cardiovascular Science and Technology: Cardiac MRI

MONDAY AM

Org by Robert Balaban, Natl Insts of Health

Cardiovascular Science and Technology: Diseases of the Cytoskeleton and Contractile Apparatus

MONDAY PM

Org by James Sellers, Natl Insts of Health

Signal Transduction

FRIDAY AM, PM

Org by Kathleen Kelly, Natl Cancer Inst

DNA Computing

FRIDAY AM

Org by Richard Lipton, Princeton Univ

Quantum Computation

FRIDAY PM

Org by Seth Lloyd, Massachusetts Inst of Tech

Structural and Molecular Basis of Cancer Vaccines

SATURDAY AM

Org by Jill O'Donnell-Tormey, Cancer Resch Inst, and Brian Quinn, Cancer Resch Inst

Chromosomal Instability as a Causal Factor in Cancer

SATURDAY PM

Org by Lawrence Grossman, Johns Hopkins Univ

Structural Biology

SATURDAY PM

Org by Michael F. Summers, Univ of Maryland -Baltimore

Advances in AIDS Research

SUNDAY AM, PM; MONDAY AM, PM

Org by Steven Wolinsky, Northwestern Univ-Med Schl

Genomics: Bacteria and Plants

SUNDAY AM

Org by William A. Haseltine, Human Genome Sciences

Genomics: Animals

SUNDAY PM

Org by William A. Haseltine, Human Genome Sciences

Reproductive and Developmental Biology: Innovations in Fertility

MONDAY AM

Org by Florence Haseltine, Natl Insts of Health, and Wayne Bardin, Population Council

Reproductive and Developmental Biology: Developmental Biology

MONDAY PM

Org by Florence Haseltine, Natl Insts of Health, and Wayne Bardin, Population Council

REGISTER FOR AMSIE'96 TODAY

Phone: (202) 326-6417

FAX: (202) 289-4021

Internet: **http://www.aaas.org**

REGISTRATION FORM – SEE PAGE 16

GENERAL

Information

Meeting Locations

Baltimore Convention Center
One West Pratt Street, Baltimore, MD 21201
AMSIE'96 activities include: Concurrent Sessions, Science Innovation, Poster Sessions, Headquarter's Office, Meeting Registration, Exhibition, Employment Exchange and Career Development Workshops.

Hyatt Regency Baltimore
300 Light Street, Baltimore, MD 21202
AMSIE'96 activities include: Plenary and Topical Lectures, and Concurrent Sessions.

Stouffer Renaissance Hotel
202 East Pratt Street, Baltimore, MD 21202
AMSIE'96 activities include: Topical Lectures, Seminars, and Concurrent Sessions.

Hotel Information

Hyatt Regency Baltimore
Conveniently located on the Inner Harbor, the Hyatt has been one of the city's foremost hotel since its opening. Connected to the hotel by Skywalk is Baltimore's famous Harborplace, the Gallery, and the Baltimore Convention Center. Other major attractions, including the National Aquarium, the Science Center, and the Imax Theater are all within easy walking distance. The Hyatt Regency offers guests every amenity for a memorable stay: two restaurants, a lounge, health club, incredible views, luxurious comfort, superb service, and more. AMSIE'96 activities located at the Hyatt Regency are Plenary and Topical Lectures and Concurrent Sessions.

Stouffer Renaissance
Awarded the prestigious AAA Four-Diamond Award and the Mobil Four-Star Award, the Stouffer Renaissance Harborplace surrounds guests with superior comforts and personalized extras. It is located on the gleaming waterfront of the Inner Harbor, just two short blocks from the Baltimore Convention Center. Amenities include bilingual concierge service, full secretarial services, 24-hour room service, a fully equipped health club, and an indoor pool. AMSIE'96 activities located at the Stouffer Renaissance will be Topical Lectures, Seminars, and some Concurrent Sessions.

Sheraton Inner Harbor
The Sheraton is located directly adjacent to the Baltimore Convention Center and just one block from the shops, restaurants, and attractions of Baltimore's scenic Inner Harbor. This deluxe high rise hotel offers first class service and prides itself on maintaining a small intimate hotel atmosphere. Each guest room is attractively designed for maximum guests comfort. A fully stocked honor bar, coffee pot, blow dryer, iron, and ironing board are just a few of the amenities in each room.

Marriott Inner Harbor
Located only two blocks from the Baltimore Convention Center, the Marriott offers a health club with an oversized indoor pool, whirlpool, sauna, and fully equipped exercise room. Excellent dining options include the Promenade, an upscale multi-purpose restaurant and the Bumpers Dance Bar featuring music of the 50s and 60s. Oversized guest rooms feature individual climate control, remote control cable TV with pay movies, AM/FM radios, and more.

Local Transportation

Airport Taxi: Cabs are available from Baltimore-Washington International Airport (BWI). Taxi stands are located on the airport's lower level near each exit. The fare from BWI to downtown Baltimore is about $15 (flat rates are illegal).
Airport Super Shuttle: The Super Shuttle departs from BWI Airport for downtown Baltimore hotels every half hour between 6:00 AM and 12:30 AM (except holidays). The price is $10 one way, and $15 round trip. For return trips to BWI, reservations are required two hours in advance. For more information call (410) 859-0800.

Rail: Both Amtrak and the local MARC trains provide rail service between BWI Airport and Baltimore's Penn Station. It is a short taxi ride from Baltimore-Penn Station to all downtown hotels. In addition, Amtrak offers convenient service directly to Baltimore-Penn Station from New York, Newark, Wilmington, Philadelphia, and Washington, DC. For Amtrak rates and schedules call: 1-800-USA-RAIL; or contact MARC at 1-800-325-RAIL.

Parking: All AMSIE'96 hotels have on-site parking. Parking rates for hotel guests range from $8 to $10 per day.

Discount Air Fares

Get discount airfares to AMSIE'96 on two major airlines. Make your reservation on either of the following airlines and save money on air fares for travel to and from Baltimore.

American Airlines: Save with special domestic zone fares (2-night stay minimum, no Saturday night requirement) or save 5% off the lowest applicable domestic coach fares – for travel to and from the Baltimore-Washington Area (including Baltimore-Washington International (BWI), National, and Dulles Airports) between February 5-16, 1996. Seats are limited and some restrictions may apply. For details and to make reservations, you or your travel agent can call:
American Airlines
1-800-443-1790 and give the American Airlines agent the AAAS Star Number: 1826AB

Continued

On-Site Registration

AMSIE'96 Registration will be located at the Baltimore Convention Center, unless otherwise indicated. Please note operational hours below.

Thurs	Feb 8, 1996	7:00 AM - 12:00 PM	Seminar Only Registration Stouffer Renaissance
		2:00 PM - 7:00 PM	General Registration
Fri-Mon	Feb 9-12, 1996	7:00 AM - 5:00 PM	General Registration
Tues	Feb 13, 1996	8:00 AM - 1:00 PM	General Registration

HOTEL FORM – SEE PAGE 17

Important Deadlines

Advance Registration: January 5, 1996

Hotel Reservation: January 5, 1996

Call for Poster Papers: November 1, 1995

U.S. Air: Save 10% off unrestricted domestic coach fares (7 day advance purchase required), 5% off the lowest applicable domestic fares, or 5% off first-class fares — for travel to and from the Baltimore-Washington Area (including, Baltimore-Washington International (BWI), National, and Dulles Airports) between February 5-21, 1996. Seats are limited and some restrictions may apply. For details and to make reservations, you or your travel agent can call;

U.S. Air:
1-800-334-8644
and give the U.S. Air agent the AAAS Gold File Number:
11120010

Outside the United States, contact your local representative for either of the airlines above.

Field Trips

The field trips described below will take participants to places in the Baltimore-Washington Area that enrich the scientific content of AMSIE'96. The trips will be kept small (as few as 20 people) so that everyone can talk with the leaders and learn from the sites. Separate registration is required, see the AMSIE'96 Registration Form. Early enrollment is advised due to space limitations. Field trips are limited to AMSIE'96 registrants and their guests. AAAS reserves the right to cancel a scheduled field trip due to insufficient registration. In the event of such cancellations, fees will be fully refunded except where noted.

Columbus Science Center
Duration: Half Day
Date: Thursday, February 8, 1996 Time: 1:00 PM - 5:00 PM
Cost: $5 AAAS Member/$7.50 All Others. No refund.
A fascinating new science center opening a few months after the meeting. AMSIE'96 has arranged a special tour that includes discussions with the center's researchers and developers about the concept, goals, and plans of the center. Tour participants will be among the first to visit this beautiful, new facility that highlights the importance of the worlds marine environment and its diversity. State-of-the-art interactive exhibits will take the group from the global to the atomic levels in organization. In addition, you will also explore the role of modern molecular biology and view the working laboratories of what is expected to be a world-class research facility.

NASA Goddard Space Flight Center
Duration: Half Day
Date: Tuesday, February 13, 1996 Time: 8:30 AM - 12:30 PM
Cost: $25 AAAS Member/$30 All Others. Price includes transportation.
In cooperation with the NASA Goddard facility a specially-developed tour is being prepared for AMSIE'96 registrants and their guests. Participants will not take the regular public tour, but instead will meet NASA staff scientists and will examine important areas of the research and development activities of this major aerospace center.

Johns Hopkins Applied Physics Laboratory
Duration: Half Day
Date: Thursday, February 8, 1996 Time: 1:00 PM - 5:00 PM
Cost: $25 AAAS Member/$30 All Others. Price includes transportation.
Special arrangements are underway for a tour of the Applied Physics Laboratory at Johns Hopkins University. Participants will have the opportunity to meet staff scientists; discuss the ongoing work of the facility; and view specific research projects.

U.S. Department of Agriculture - Beltsville Agricultural Research Center
Duration: Half Day
Date: Tuesday, February 13, 1996 Time: 12:30 PM - 5:00 PM
Cost: $25 AAAS Member/$30 All Others. Price includes transportation.
Modern agriculture is rapidly moving towards low-input, sustainable methods that are environmentally responsible. This specially designed tour of a major USDA facility will provide the opportunity for participates to examine the newest and emerging agricultural methods and to view the ongoing research in sustainable agriculture.

Special Events

AAAS Public Science Day
Thursday, February 8, 1996
This year's Public Science Day will bring together thousands of Baltimore citizens, especially K-12 students and local science resources. Participants will enjoy a full day of events that include special programs, activities and field trips that emphasize the importance of science and technology in our daily lives. Students will learn about scientific careers and course selections; they will participate in technologies of the future through workshops and activities conducted by scientists, such as hands-on science and mathematics experiments; scientists will be linked to classrooms throughout the state via interactive video; and grandparents will take preschoolers to the National Aquarium to see how the fish and other animals are cared for and trained. The grand finale includes an exciting Family Night program.
For more information contact: Amie Hubbard at (202) 326-6760; or on the internet at: ahubbard@aaas.org.

American Junior Academy of Science (AJAS)
The 1996 AJAS Meeting will be held in conjunction with AMSIE'96. Over the course of four days, the AJAS will showcase the work of promising future scientists from across the country. These premier high school students will share their award winning work in oral and poster presentations. Students will participate in a host of academic, social and cultural events including visits to the Smithsonian Environmental Research Center, Maryland Science Center, National institute on Drug Abuse Addiction Research Center, University of Maryland Center of Marine Biotechnology, and the Baltimore National Aquarium. The grand finale includes an Awards Banquet where students will be recognized for their accomplishments followed by a moonlight cruise on the Chesapeake Bay.
For more information contact: Gloria J. Takahashi at (213) 744-3384 or in California, call (818) 333-2173

Barrier-Free Environment

Accommodations for people with disabilities are provided upon request at all general lectures and other sessions. Services include interpreters for the deaf or hearing impaired, audiotaped highlights for the blind or visually impaired, and mobility assistance. In addition, a resource room for people with disabilities will be available in Room 315 of the Baltimore Convention Center. Please make sure to check the special needs box on the registration form. AAAS will contact you before the meeting.

AAAS Employment Exchange

The AAAS Employment Exchange/Job Fair and the Career Development Seminars and Workshops will take place at the Baltimore Convention Center.

Career Development Seminars and Workshops:
February 10 -12, 1996
8:30 AM - 5:30 PM

Employment Exchange/Job Fair
February 12 -13, 1996
10:00 AM - 4:00 PM

REGISTRATION FORM – SEE PAGE 16

ADVANCE REGISTRATION FORM

Advance Deadline: January 5, 1996 *Copy this form for a friend*

The AAAS Annual Meeting & Science Innovation Exposition* • February 8-13, 1996 • Baltimore, MD

I. REGISTRANT INFORMATION *(Please type or print clearly; allow 40 characters per line for name and institution)*

First Name_____ Last Name_____

Institution/Company_____

Mailing Address_____ City/State/Zip_____

Country_____ Internet Address_____

Daytime Phone Number_____ FAX Number_____

AAAS Membership Number_____ AAAS Primary Section_____

II. PATRON/SPECIAL NEEDS/SPECIAL RATES VERIFICATION

❑ Check here if AAAS Patron ❑ Check here if special needs due to disability

*To qualify for Student or *Special registration fees, please complete the appropriate line below:*

REGISTER NOW & SAVE!
Late & On-Site Fees Are $20-$50 Higher!

❑ Student: Institution Name:_____ Level:_____

❑ Postdoc: Chairperson's Name:_____ Phone Number:_____

❑ K-12 Teacher: Principal's Name:_____ Phone Number:_____

❑ Retired: Year of Retirement_____ Year of Birth:_____

III. ADVANCE REGISTRATION FEES (*Special = Retired, K-12 Teacher, Postdoctorals.)

A. CONFERENCE — AAAS Member / Non-Member

AMSIE '96 Passport: Unlimited access – Full entry to all sessions - General Meeting, Science Innovation, all Seminars; plenary and topical lectures, exhibition hall and workshops.

BEST BUY!

	AAAS Member	Non-Member
Registrant	❑ $195	❑ $245
Special*[1] Registrant	❑ $150	❑ $180
Student[1] Registrant	❑ $110	❑ $130

General Meeting Symposia: Entry to the General Meeting symposia, plenary and topical lectures, exhibition hall and workshops only.

	AAAS Member	Non-Member
Registrant	❑ $155	❑ $205
Special*[1] Registrant	❑ $85	❑ $115
Student[1] Registrant	❑ $30	❑ $50

Science Innovation Symposia: Entry to the Science Innovation symposia, plenary and topical lectures, exhibition hall and workshops only.

	AAAS Member	Non-Member
All Registrants	❑ $100	❑ $155

* Special = Postdoc, retired, and K-12 Teacher

B. EACH SEMINAR — AAAS Member / Non-Member

Please specify seminar below.

	AAAS Member	Non-Member
All Registrants	❑ $100	❑ $155

❑ Origins Seminar
❑ Living with the Internet Seminar

C. ONE DAY ONLY — AAAS Member / Non-Member

Please specify day below.

	AAAS Member	Non-Member
All Registrants	❑ $100	❑ $155

❑ Thurs ❑ Fri ❑ Sat ❑ Sun ❑ Mon ❑ Tues

D. MEMBERSHIP[2]

If you are not a AAAS member, you can join now by checking the appropriate box below – and take advantage of the discounted member registration fees. You'll also get a year's subscription (51 issues) to the journal SCIENCE at the lower 1995 rate.

	USA	Canada	International
Member	❑ $97	❑ $160.50	❑ $190
Student Member	❑ $50	❑ $110.21	❑ $143
Postdoc Member	❑ $72	❑ $133.75	❑ $165
Retired Member	❑ $53	❑ $113.42	❑ $146

E. OPTIONAL

	AAAS Member	Non-Member	No. of People
Exhibit Only Pass	❑ $25	❑ $30	
Field Trips: Please specify field trip(s) and number of people. Prices are per person.			
Columbus Sci Ctr	❑ $5	❑ $7.50	_____
Check date for Columbus Center:	❑ *2-8-96*	❑ *2-13-96*	
NASA Goddard	❑ $25	❑ $30	_____
Johns Hopkins	❑ $25	❑ $30	_____
U.S.D.A	❑ $25	❑ $30	_____

IV. PAYMENT[3]

Check appropriate boxes under section III A, B, C or D and write in Advance Fees below.

A. CONFERENCE FEES:

AMSIE '96 Passport (Unlimited Access).....$_____

***Note:** Seminars and Science Innovation are free to AMSIE'96 Passport registrants, but you must indicate below the program(s) you plan to attend:*

❑ Origins Seminar ❑ Living with the Internet Seminar ❑ Science Innovation

General Meeting Symposia.....$_____

Science Innovation Symposia.....$_____

B. SEMINARS

❑ Origins Seminar.....$_____

❑ Living with the Internet Seminar.....$_____

C. ONE DAY ONLY.....$_____

D. MEMBERSHIP DUES (if joining now).....$_____

E. OPTION FEES

Exhibit Only Pass.....$_____

Field Trip Total.....$_____

Total Amount.....$_____

Payment Method: ❑ Check enclosed ❑ Original purchase order enclosed

Charge to my: ❑ VISA ❑ MasterCard ❑ AmEx *(no other cards accepted)*

Credit Card Number:_____

Signature:_____ Exp. Date:_____

V. HOW TO REGISTER

Where Science Comes To Life

Phone or FAX your registration today. **ADVANCE REGISTRATION DEADLINE 1/5/96**

PHONE: (202) 326-6417 *(Credit card payments only.)*

FAX: (202) 289-4021 *(Credit card payments only.)*

Avoid duplicate billing - do NOT mail hard copy if you register via FAX.

MAIL: AAAS • Meetings Dept. • P.O. Box 630285 • Baltimore, MD 21263

Important Notes:

[1] Students, postdocs, K-12 teachers, and retired members must complete the verification information to be accepted at the special registration rates.

[2] Membership dues indicated hereon are the 1995 rates. Although dues will increase on January 1, 1996, the 1995 rates are guaranteed through February 8, 1996 for registration of the annual meeting. $50 of dues plus international postage are allocated to SCIENCE. Please allow up to 4 weeks for receipt of your first issue of SCIENCE. Canadian rates include GST #125488122.

[3] Cancellations must be received in writing by **January 5, 1996.** No refunds will be made for cancellations received after this date. Refunds are subject to a $25 cancellation charge and will be processed after the meeting.

[4] Checks must be in United States currency and must be payable on a United States bank. SCI-1096

American Association for the Advancement of Science

HOUSING FORM

Deadline: January 5, 1996

The AAAS Annual Meeting & Science Innovation Exposition • February 8-13, 1996 • Baltimore, MD

Instructions: Please complete section 1, 2 and 3 below (if not legible, form will not be processed). All reservations must be made through the Baltimore Area Convention and Visitors Association (BACVA) Housing Bureau by mail or fax. NO PHONE REQUESTS WILL BE ACCEPTED. The bureau will acknowledge receipt of your reservation within 10 days by mail. No fax acknowledgments possible. Room confirmation will be mailed by the hotel. Confirmations will be sent to the individual below. **Reservations must be made by January 5, 1996**. After this date, hotel space and convention rates may not be available.

I. Room Request *Please use a separate form for each room request. (This form may be copied.)*

Name: _____
Last Name First Name

Institution: _____
(if part of mailing address)

Address: _____

City: _____ **State:** _____ **Zip:** _____

Country: _____

Daytime Telephone Number: ( ) _____

Room Request: ❑1 bed, 1 person ❑ 1 bed, 2 people ❑ 2 beds, 2 people ❑ 2 beds, 3 people ❑ 2 beds, 4 people

Special Needs: ❑ Wheelchair accessible room ❑ Non-smoking room ❑ Other _____

Arrival Date: _____ **Arrival Time:** _____

Departure Date: _____ **Departure Time:** _____

II. Hotel Preference

Please list all four choices by code. All hotel rates are subject to 12% sales tax.

1. _____ **2.** _____ **3.** _____ **4.** _____

HOTELS	CODE	Single	Double	Each Additional Person
Hyatt Regency Baltimore*	**HYATT**	$ 118	$ 136	$25
Stouffer Renaissance*	**STOUF**	$ 126	$ 141	$15
Sheraton Inner Harbor	**SHERI**	$ 115	$ 135	$15
Marriott Inner Harbor	**MARIH**	$ 115	$ 130	$15

Room rates include $1 per night surcharge to help defray AAAS meeting costs.
** Meeting activities will take place in the Baltimore Convention Center, the Hyatt Regency and the Stouffer Renaissance.*

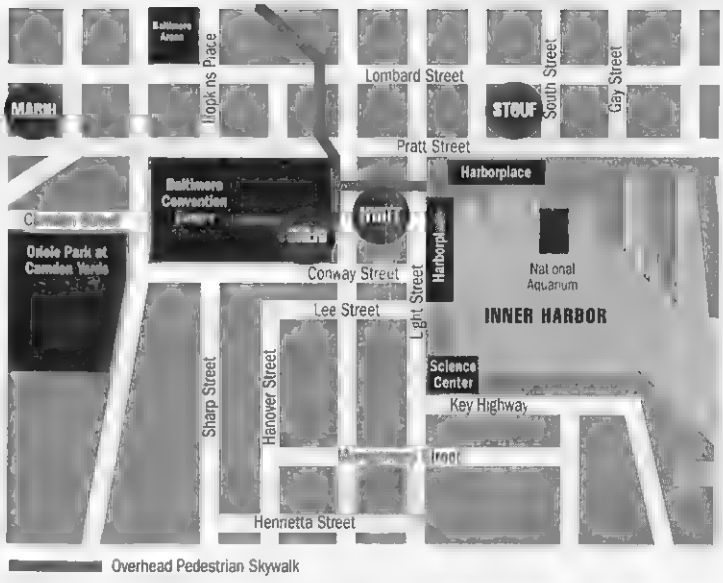
LIST FULL NAME OF ADDITIONAL ROOM OCCUPANTS:

1. _____

2. _____

3. _____

4. _____



III. Method of Payment

A credit card number or room deposit of $100 must accompany this form. Forms received without credit card information or check will not be processed. BACVA accepts no liability once deposits are transferred to the assigned hotel.

Please check one: ❑ VISA ❑ MasterCard ❑ American Express ❑ Discover ❑ Diners Club ❑ Check Enclosed *(payable to BACVA Housing Bureau)*

Credit Card Number #:

|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|

Expiration Date: _____

Signature: _____

Changes/cancellations: reservation changes should be made directly with your assigned hotel. Cancellations must be made through your assigned hotel no later than 72 hours prior to arrival to receive a refund of your deposit.

IV. Mailing Instructions

Where Science Comes To Life

MAIL THIS FORM AND DEPOSIT TO:
BACVA Housing Bureau (AMSIE '96)
100 Light Street, 12th Floor, Baltimore, MD 21202
FAX: (410) 659-7313 (credit card reservations only)
DO NOT MAIL THIS FORM TO THE HOTELS.

American Association for the Advancement of Science

PARTIAL LISTING AS OF PRESS TIME

Exhibitors

FEBRUARY 8-13, 1996
BALTIMORE
CONVENTION CENTER
BALTIMORE, MARYLAND

Academia Book Exhibits

American Association for the Accreditation of Laboratory Animal Care

American Association of Pharmaceutical Science

American Association of University Presses

American Chemical Society

American Institute of Physics

The Boeing Company

Chlorine Chemistry Council

Cornell Theory Center

Dover Publications, Inc.

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Ecumenical Roundtable on Science Technology and the Christian Faith

Engineering Technology Center

Graduate Women In Science, Inc.

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Heldref Publications

Howard Hughes Medical Institute

Island Press

Johns Hopkins University Press

Media Cybernetics

National Library of Medicine-SIS

National Science Foundation

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POSITIONS OPEN

ASSOCIATE DIRECTOR FOR RESEARCH

The Markey Cancer Center at the University of Kentucky is seeking candidates at the Associate or full Professorial level to serve as **ASSOCIATE DIRECTOR** for Research. This individual will coordinate cancer related research activities involving more than 150 faculty from 24 departments and six colleges at the University of Kentucky, providing leadership in the definition, articulation, and promotion of interdisciplinary research programs of the center. The successful applicant should have a demonstrated record of accomplishment in a cancer related field of research, an interest in fostering the development of new projects and collaborations in all areas of cancer research, and leadership abilities. The applicant will receive a primary appointment in an appropriate department of the College of Medicine of the University of Kentucky. This position carries substantial opportunities for professional growth and development while coordinating a rapidly expanding program of excellence. The Markey Cancer Center is a newly developing comprehensive center for cancer research, education, patient care, and community outreach programs serving the state of Kentucky and the surrounding region. Special features include eight core research facilities: Macromolecular Structure Analysis, Flow Cytometry, Transgenic Cell Production, Specialized Animal Care, Biostatistics, Tissue Procurement and Banking, a Magnetic Resonance Imaging and Spectroscopy Center, and a Clinical Research Office. Applicants with the Ph.D. or M.D. degree should send their curriculum vitae, copies of reprints, a brief summary of research accomplishments, future research plans, and three letters of reference to: **Dr. Lewis Kelly, Administrator, Associate Director Search Committee, Markey Cancer Center, 800 Rose Street, Lexington, KY 40536-0093.** *The University of Kentucky is an Affirmative Action/Equal Opportunity Employer*

TENURE-TRACK ASSISTANT PROFESSOR TUFTS UNIVERSITY CONSERVATION BIOLOGIST

Conservation Biologist. The Biology Department invites applications from ecologists addressing questions related to biological diversity and conservation at the population or community level. The position will begin in fall 1996. Applicants must have a Ph.D.; teaching and postdoctoral experience desirable. Research should emphasize community structure and ecological interactions in any terrestrial or aquatic habitat. Duties will include participation in university wide environmental programs, and teaching introductory and upper-level courses in environmental and conservation biology. Candidates will be expected to develop an active research program involving undergraduate and graduate students in biology. Applications should include curriculum vitae, reprints of up to three publications, and statements of 1) present and future research plans, 2) teaching interests, and 3) interest in interdisciplinary environmental programs. Please send application and have three letters of recommendation sent to: **Professor Jan A. Pechenik, Biology Search Committee, Tufts University, Medford, MA 02155.** Completed applications must be received by December 18, 1995. *Tufts University is an Equal Opportunity/Affirmative Action Employer. Women and minorities are encouraged to apply*

ENVIRONMENTAL PLANT BIOLOGIST

The Department of Biology, University of South Florida (USF), anticipates a nine month tenure-track, **ASSISTANT PROFESSOR** position starting fall 1996. Candidates must have a Ph.D., postdoctoral experience preferred, and be able to teach undergraduate courses in ecology, evolution, or genetics, including a team-taught course in environmental science, and graduate courses in specialty areas. Supervision of M.S. and Ph.D. students is expected. Areas of particular interest are physiological, molecular, or landscape ecology, or conservation or evolutionary biology. USF, the second largest university in Florida, is a comprehensive research/teaching university. The Department has 32 faculty members and over 65 ecologically oriented graduate students. Applicants should forward a letter of intent, curriculum vitae, reprints, statements of research interests and teaching goals, and arrange for three letters of recommendation to be received by January 15, 1996 to: **Environmental Plant Biologist Search, Department of Biology, University of South Florida, Tampa, FL 33620-5150.** *USF is an Equal Opportunity/Affirmative Action/Equal Access Institution. For disability accommodation* call Dr. Romeo (813-974-3250) a minimum of five working days in advance.

POSITIONS OPEN

ASSISTANT PROFESSORS CHEMISTRY AND PHYSICS Southeastern Louisiana University (SLU)

The Department of Chemistry and Physics at Southeastern Louisiana University invites applications for three tenure-track **ASSISTANT PROFESSORS** of Chemistry available August 1996. Qualifications: undergraduate degree in chemistry and Ph.D. in either chemistry or biochemistry from an accredited university. Preference will be given to applicants in analytical, physical, and biochemistry. Applicants must possess a strong commitment to undergraduate teaching, not only in their area of specialization, but also in introductory and service courses, and be prepared to develop a vigorous, independent research program that will attract outside funding. Additional emphasis will be placed on instrumental and laboratory skills. Responsibilities: teach undergraduate courses in chemistry and/or biochemistry; develop active research program involving undergraduates in area of espertise, establish record of scholarly activity and publications; serve as academic adviser to chemistry majors; participate in department, college, and university service. Salary, commensurate with experience and qualifications. Applications will be accepted until the positions are filled Send letter of application, a complete curriculum vitae including telephone numbers and addresses, and a listing of all publications, copies of all transcripts (originals required upon employment), three current letters of recommendation, a brief statement of teaching experience and philosophy, and a brief statement of research interests to: **Dr. Linda Munchausen, Department of Chemistry and Physics, Southeastern Louisiana University, SLU 878, Hammond, LA 70402.** *SLU is an Affirmative Action/Americans with Disabilities Act/Equal Employment Opportunity Employer.*

POPULATION GENETICIST

The Division of Biological Sciences at the University of Missouri-Columbia (MU) continues a multi-year hiring program with a tenure-track **ASSISTANT PROFESSORSHIP** for a population geneticist with a strong background in evolutionary theory. The ideal candidate will employ theoretical or molecular techniques in the study of natural or experimental populations with an emphasis on the mechanisms of evolutionary change. Opportunities for collaboration abound: MU has strong interdisciplinary programs in genetics and molecular biology, and the Division has major research programs in the evolution of behavior and plant population biology. A highly competitive salary, excellent start-up package, modern research laboratories, and an active graduate program provide an extremely supportive environment Candidates should have postdoctoral experience, demonstrated potential for creative research, and a commitment to teaching at the undergraduate and graduate levels. Send curriculum vitae, reprints of publications, statement of research and teaching interests, and three letters of reference to:

Dr. John David
Division of Biological Sciences
105 Tucker Hall
University of Missouri-Columbia
Columbia, MO 65211

Applications should be received by December 1, 1995 to receive full consideration. *MU is an Equal Opportunity/Affirmative Action/Equal Access Institution. We are firmly committed to fostering ethnic and racial diversity on our faculty and strongly encourage applications from minorities and women.*

VERTEBRATE BIOLOGIST/ECOLOGIST

The Department of Biology of Carroll College, a coeducational, small, Catholic, liberal arts college, invites applications for a full-time (academic year), tenure-track **ASSISTANT PROFESSOR** beginning fall 1996. Ph.D. is required. Teaching responsibilities include Ecology, Comparative Anatomy, Life Science for non-majors, and a portion of a team-taught Introductory Biology course for majors. Candidates should be committed to undergraduate teaching with high academic standards, research and thesis programs, and student advising. Aggressive grant seeking and research not required Send cover letter, curriculum vitae, transcripts, and three letters of recommendation by December 1, 1995 to: **Dr. Bruce Busby, Academic Vice President, Carroll College, 1601 North Benton Avenue, Helena, MT 59625-0002.** *Equal Employment Opportunity.*

FACULTY POSITION IN BACTERIAL PATHOGENESIS

The Department of Microbiology at the College of Physicians and Surgeons of Columbia University is soliciting applications for a tenure-track faculty position at the level of **ASSISTANT PROFESSOR**. Applicants should have a strong background in prokaryotic genetics and experience in an area related to the interaction of pathogens with host cells. Applicants should have demonstrated a high level of academic achievement and the potential for developing an active research program. The Department has an active graduate program, supported by a training grant, and all faculty are expected to maintain a strong commitment to graduate education. Substantial start-up resources are available. Interested individuals should send a curriculum vitae and a statement of research interests and should also arrange to have three letters of reference sent to: **The Microbiology Search Committee, Department of Microbiology, College of Physicians and Surgeons, Columbia University, 701 West 168th Street, New York, NY 10032. Email: horan@cumbib.bmb.columbia.edu.**

Columbia University is an Affirmative Action/Equal Opportunity Employer.

TWO ASSISTANT PROFESSORS

The Department of Biological Sciences of the University of Arkansas, Fayetteville is searching for a **PLANT PHYSIOLOGIST** and a **PLANT GENETICIST** for tenure-track appointments beginning 15 August 1996. Preference for the geneticist position will be given to candidates using molecular approaches in the areas of developmental or population genetics. Candidates must have a Ph.D. and postdoctoral experience. Appointees must be prepared to participate in freshman, upper, and graduate-level courses, and to establish an independent research program that incorporates Master's and Ph.D. students. Salary and start-up are competitive. Review of applicants begins December 1, 1995 and continues until the positions are filled. Submit letter of application, curriculum vitae, statements of research and teaching interest, reprints, and have three letters of recommendation sent to: **Dr. Richard L. Meyer, Chair, Plant Physiologist Search Committee, or Dr. Edwin B. Smith, Chair, Plant Geneticist Search Committee, Department of Biological Sciences, SCEN-629, University of Arkansas, Fayetteville, AR 72701.** *The University of Arkansas is an Equal Opportunity/Affirmative Action Institution. Women and Minorities are encouraged to apply.*

RESEARCH FACULTY POSITION

The Division of Medical Oncology, Department of Medicine, at the University of Texas Health Science Center at San Antonio (UTHSCSA) has a Research **FACULTY POSITION** available in its nationally-recognized and NIH-funded breast cancer program. Participation by the investigator in existing research areas including mechanisms of tamoxifen resistance, growth factors in breast cancer, oncogenes and suppressor genes, estrogen receptor variants, signal transduction, and transgenic mouse models, as well as development of an independent research area are desired. Minimum requirements are a Ph.D. degree in molecular and/or cell biology, postdoctoral training, and one to two years of experience preferred. *The position requires U.S. citizenship or permanent residency status.* Appointment will be at the Instructor or Assistant Professor level. Send curriculum vitae, letter of research interests, and names of three references to: **C. Kent Osborne, M.D., Department of Medicine/Medical Oncology, UTHSCSA, 7703 Floyd Curl Drive, San Antonio, TX 78284-7884.** *The UTHSCSA is an Equal Opportunity/Affirmative Action Employer.*

ASSISTANT PROFESSOR, tenure-track. Primary teaching responsibilities in molecular biology and developmental biology. Other teaching responsibilities include participation in core courses. Requirements: Ph.D. by September 1996, demonstrated teaching ability, broad training in biology, commitment to undergradute teaching and research, and an active research record. Send curriculum vitae, copies of transcripts, copies of recent publications, and at least three references by January 15, 1996 to: **V. Sullivan, Department of Biology, University of Michigan-Flint, Flint, MI 48502-2186.** This campus is a 6,300 student, urban regional unit of the University of Michigan *and is a non-discriminatory/Affirmative Action/Equal Access Employer that specifically invites women and minorities to apply.*

BIOORGANIC/BIOMEDICAL CHEMISTRY
University of California-San Francisco (UCSF)

The Departments of Cellular and Molecular Pharmacology and Pharmaceutical Chemistry at UCSF invite applications from uniquely qualified individuals with research interests at the chemistry/biology interface. We anticipate filling up to two tenure-track positions at the **ASSISTANT PROFESSOR** level, but applications from more senior candidates may also be considered. Candidates should have a strong background in synthetic organic chemistry, and the ability and desire to use chemical approaches to study contemporary problems of biomedical interest. To apply please submit a curriculum vitae, including a list of publications and a brief outline of proposed research projects, and arrange to have three letters of recommendation forwarded. The deadline for applications is January 10, 1996. All application materials should be sent to: **Faculty Search Committee, Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA 94143-0450.** *Women and members of other underrepresented groups are particularly encouraged to apply.*

ASSISTANT PROFESSORS

The Department of Zoology and Physiology at Louisiana State University anticipates two tenure-track **ASSISTANT PROFESSOR** positions, "Pending Final Approval." The openings, starting August, 1996, are in any area of (1) Vertebrate Physiology, but with special consideration given to those who work in the areas of endocrinology or neurobiology using non-mammalian models, and (2) Cell Biology, but with special consideration given to those who use molecular techniques in conjunction with other approaches to study cytoskeletal regulation/molecular motors, signaling events or trafficking, protein targeting/ modification. Ph.D. or equivalent degree in a biological science and postdoctoral experience required. Successful applicants are expected to develop a strong, independent research program with extramural support and have a commitment to excellence in undergraduate and graduate instruction. Candidates should submit a statement of research interests and teaching philosophy, curriculum vitae, and the names of three references by December 5, 1995, or until the position is filled, to: **Chairman, Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803-1725.** *LSU is an Equal Employment Opportunity/Affirmative Action Employer.*

Monmouth College seeks applications for two tenure-track **ASSISTANT PROFESSOR** positions in biology beginning fall 1996. Candidates with a Ph.D. and a strong commitment to undergraduate teaching in a liberal arts setting are encouraged to apply. Participation in general education courses and supervision of undergraduate research projects is expected. Candidates with interdisciplinary teaching experience preferred. Two positions: a Botanist and a Physiologist, each to teach at least three of the following six courses: Botany, Animal Physiology, Cell Biology, Mendelian Genetics, Microbiology, Molecular Biology. Opportunity to develop upper-level courses in specialty and/or courses to enhance our innovative general education program. Skills and experience to augment our Environmental Science program are desirable. Send letter of application, curriculum vitae, and at least three professional references with telephone numbers to: **Michael McNall, Director of Personnel, Monmouth College, 700 East Broadway, Monmouth, IL 61462.** Formal review of applications will begin December 15, 1995, and continue until positions are filled. *Monmouth College is an Equal Opportunity/Affirmative Action Employer and encourages applications from women and minority candidates.*

TWO TENURE-TRACK POSITIONS

1) Plant Physiological Ecologist to teach botany, plant physiology, and general ecology. 2) Comparative Animal Physiologist to teach human physiology and anatomy, pathophysiology for nurses, and specialty courses. **ASSISTANT PROFESSORSHIPS**; Ph.D. required. Available September 1, 1996. Application deadline December 1, 1995. For detailed descriptions and instructions contact: **Department of Biology, California State University, 9001 Stockdale Highway, Bakersfield, CA 93311. Telephone: 805-664-3089; FAX: 805-664-2040; Email: epedroza@academic.csubak.edu.** *Affirmative Action/Equal Opportunity Employer.*

ENVIRONMENTAL BIOCHEMIST

The Biology Department at Rensselaer Polytechnic Institute invites applications for a tenure-track faculty position at **ASSISTANT** or **ASSOCIATE PROFESSOR** level, to start September 1996, from biochemists utilizing a combination of molecular genetic and biochemical approaches for research in an area related to the environment, such as toxicology, xenobiotics, aquatic biology, or water-born diseases. Development of an independent research program is expected. Requirements include two years of postdoctoral research experience or equivalent, and ability to teach undergraduate and graduate courses in biochemistry, molecular biology, and environmental science. Send curriculum vitae, statement of research plans and teaching interests, publication list, and names of three references, by December 15, 1995 to: **Prof. Joyce Diwan, Search Committee Chair, Biology Department, Science Center, Rensselaer Polytechnic Institute, Troy, NY 12180-3590.** *Rensselaer is an Affirmative Action/Equal Opportunity Employer. Women and minorities are encouraged to apply.*

ASSISTANT PROFESSOR
Molecular/Developmental Biology

The University of Rochester anticipates an opening for an **ASSISTANT PROFESSOR** of Biology. Applicants must have demonstrated outstanding potential for independent work in the area of Molecular or Developmental Biology. The Biology department consists of 22 faculty who employ genetic and molecular approaches to problems in cell, molecular, and developmental biology; evolutionary biology; and ecology. Additional faculty with allied interests are located in the adjacent Medical School. Applicants should submit before December 1, 1995, a curriculum vitae, bibliography, description of research and teaching interests, and should request three references to send letters of recommendation to: **Chair, Molecular/Developmental Search Committee, Department of Biology, University of Rochester, Rochester, NY 14627.**

The University of Rochester is an Affirmative Action/Equal Opportunity Employer.

FACULTY POSITIONS
Department of Biochemistry, Molecular Biology, and Cell Biology
Northwestern University

The Department of Biochemistry, Molecular Biology, and Cell Biology seeks outstanding candidates for full time faculty appointments at the junior or senior level. We particularly seek applicants in the areas of signal transduction, cell cycle regulation, macromolecular sorting, immune response, mammalian developmental genetics, yeast genetics, and structural biology. Candidates should submit a curriculum vitae, a list of publications, statements of research accomplishments and future research objectives, and letters of reference from three persons knowledgeable of the candidate's research and teaching abilities, to: **Chairman, Faculty Search Committee, Department of Biochemistry, Molecular Biology, and Cell Biology, 2153 North Campus Drive, Evanston, IL 60208-3500.** To ensure full consideration, complete applications should be received by December 31, 1995.

Women and minority scientists are especially encouraged to apply. Northwestern University is an Affirmative Action/Equal Opportunity Employer.

NMR FACULTY POSITION

The Biophysics Department of the University of Rochester Medical Center is seeking applicants for a tenure-track faculty position in high resolution NMR spectroscopy as applied to structural biology. Currently, Biophysics has two macromolecular crystallographers, one NMR spectroscopist, and modern x-ray and NMR facilities, including a new fully equipped 600 MHz spectrometer. While rank and salary will be commensurate with experience, the search is targeted at the **ASSISTANT PROFESSOR** level. Send complete curriculum vitae, future research plans, and the names and addresses of three references to: **W. Bernhard, Chair, NMR Search Committee, Biophysics Department, University of Rochester Medical School, Rochester, NY 14642-8408. Email: wabern@biophysics.rochester.edu.** Review of applications will begin December 8, 1995 and continue until the position is filled. *The University of Rochester is an Affirmative Action/Equal Opportunity Employer.*

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POSITIONS OPEN

ASSISTANT PROFESSOR
Signal Transduction/Protein Structure

The Division of Signal Transduction at Beth Israel Hospital seeks to hire an **ASSISTANT PROFESSOR** with expertise in solving the structures of signaling proteins. Send résumé and the names of three referees to: **Lewis Cantley, Division of Signal Transduction, Warren Alpert 152, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115.** *Women and minority candidates are encouraged to apply.*

ENVIRONMENTAL SCIENTIST
ASSISTANT PROFESSOR

The Environmental Science Program of DePaul University seeks to fill a full-time, **TENURE-TRACK** position. Areas of interest include applied ecology or conservation biology. The successful candidate will need to make a significant commitment to undergraduate education, including teaching courses to non-majors. An active research program is expected and supported. A letter indicating interest in the position, your teaching philosophy, and possible research interests should be sent, along with a curriculum vitae, to: **The Program Director, Environmental Science Program, DePaul University, 2320 North Kenmore, Chicago, IL 60614-3298.** The review and selection process will begin on December 15, 1995, although applications received after that date may be considered. *DePaul University practices Equal Opportunity in Employment and Education.*

Biology: Cornell College, a private undergraduate liberal arts college, invites applications for a **TENURE-TRACK** appointment in its Department of Biology. Responsibilities include teaching introductory biology, ecology, seminar in evolution, and upper-level courses in botany (plant physiology, plant taxonomy/systematics, and biological problems). The successful candidate will be expected to teach introductory level courses for majors and to develop introductory level courses for non-majors. Appointment at the assistant professor level to begin in the fall of 1996. Shared appointment will be considered. Ph.D. required; postdoctoral experience and college teaching experience preferred. Cornell College has attracted national attention for its distinctive academic calendar under which faculty teach and students take one course at a time in month-long terms. The College is committed to excellence in teaching and encourages interdisciplinary interests among its faculty. Candidates for this position should have the ability to develop a research program that incorporates undergraduate students. Send letter, curriculum vitae, and three letters of reference to: **Ms. Ann Opatz, Assistant for Academic Recruitment, Cornell College, 600 First Street West, Mount Vernon, IA 52314-1098.** Formal consideration of applications begins December 1, 1995. *Cornell College is an Equal Opportunity/Affirmative Action Employer and encourages applications from women and minority candidates.*

Chemist. Sarah Lawrence College, a liberal arts college dedicated to individualized education, is recruiting a broadly trained individual for a **TENURE-TRACK** position in general or organic chemistry, beginning fall 1996. Working closely with students on a daily basis is essential. Candidates will also be expected to develop additional courses of interest to liberal arts undergraduates. Ability to teach courses in related areas, such as environmental chemistry, is desirable. Send curriculum vitae or résumé, three letters of recommendation, and statement of teaching interests by December 15, 1995 to: **Dr. Gene Rinchik, Sarah Lawrence College, Bronxville, NY 10708.** *Equal Opportunity Employer. Applications from women and minorities are encouraged.*

PLANT TAXONOMY/BIOLOGY

TENURE-TRACK position commencing August 1996. Earned doctorate in plant systematics or closely related area required; knowledge of mid-western and tropical flora and teaching experience with evidence of superior teaching performance preferred. Rank and salary commensurate with qualifications. Teaching responsibilities include: vascular plant taxonomy, general botany, and general biology. Send curriculum vitae, letter of application, and three letters of recommendation to: **Dr. J. Larry Martin, Dean, School of Arts and Sciences, Missouri Southern State College, 3950 East Newman Road, Joplin, MO 64801-1595.** Questions may be addressed to: **Dr. John Messick, Telephone: 417-625-9617; Email: messic@vm.mssc.edu.** Closing date: December 31, 1995. *Equal Opportunity/Affirmative Action Employer.*

HEAD, Department of Forestry and Wildlife Management, University of Massachusetts at Amherst. Includes the administration of teaching, research, and public outreach activities for a productive multidisciplinary program in natural resources. Candidates must have a demonstrated record of accomplishment in scholarly work, a talent for organization, and an ability to work with faculty representing a wide variety of natural resource and life science disciplines. A doctorate with at least one advanced degree in natural resources, and administrative experience, are required. The position is available July 1, 1996, subject to funding. Applications should be received by January 19, 1996 to receive priority consideration. Send nominations or applications (résumé, letter of intent, and names of three references) to: **David B. Kittredge, Chair, Search Committee for the Head, Department of Forestry and Wildlife Management, Holdsworth Natural Resources Center, University of Massachusetts, Amherst, MA 01003. Telephone: 413-545-2943; FAX: 413-545-4358; Email: dbk@forwild.umass.edu.** *The University of Massachusetts is an Affirmative Action/Equal Opportunity/Americans with Disabilities Act Employer.*

Tenure-track faculty position available in structural biology in the Department of Biochemistry at the rank of **ASSISTANT** or **ASSOCIATE PROFESSOR** beginning July 1, 1996. We seek a candidate who will develop an active teaching and research program in the application of x-ray diffraction analysis to problems in the field of structural biology. Candidates should have a Ph.D. or M.D. degree, a minimum of two years of postdoctoral experience, with evidence of significant research accomplishments, and, for appointment at the associate level, a demonstrated ability to attract extramural funding. Send curriculum vitae, a short description of research plans, and the names and telephone numbers of three references to: **Dr. Marilynn S. Doscher, Department of Biochemistry, Wayne State University School of Medicine, 540 East Canfield Avenue, Detroit, MI 48201.** *Wayne State University is an Affirmative Action/Equal Opportunity Employer.*

Research Ecologist, **TENURE-TRACK,** University of Puerto Rico. We are seeking an individual to serve as scientific director of the El Verde Field Station, one of two focal study sites in the Luquillo Long-Term Ecological Research Program. The successful candidate will be expected to manage the field station and supervise its personnel, coordinate activities of visiting researchers, and develop a strong research program of his or her own. Area of research expertise is open but preference will be given to candidates studying invertebrate ecology. Preference will also be given to candidates with an ability to communicate in Spanish. Send curriculum vitae, statement of research interests, and names of three references by 30 November 1995 to: **Robert B. Waide, Terrestrial Ecology Division, P.O. Box 363682, San Juan, PR 00936.**

ASSISTANT PROFESSOR, Chemistry, tenure-track, starting August 1996. Must have completed Ph.D. by July 1, 1996. Approved since 1957, department awards ACS-certified degrees in chemistry, chemistry with biochemical emphasis, and interdisciplinary degree in biochemistry. Four full-time faculty utilize modern instrumentation such as GC, HPLC, AA, FTIR, and UN/Vis and fluorescence spectrophotometers. Will teach physical and analytical chemistry, share responsibilities in introductory chemistry. Specialty in physical or analytical chemistry, practical experience with a wide variety of computer-controlled instrumentation. Collaborative research with undergraduates expected. Chair: **Dr. Linda Hodges, Agnes Scott College, Campbell Hall.**

VISITING PROFESSOR (rank open), Women in Science, during 1997–98, full-time or half-time. Responsibilities include teaching and faculty development seminars. Salary commensurate with rank and qualifications. Desirable opportunity for Faculty on Sabbatical. Candidates for junior rank must have completed Ph.D. by July 1, 1997. Chair: **Dr. Gail Cabisius, Box 736.**

Application deadline November 30, 1995. Mail letter of application describing teaching philosophy, research plans, curriculum vitae, and names, addresses, and telephone numbers of three professional references to: **Search Chair, Agnes Scott College, 141 East College Avenue, Decatur, GA 30030-3797.** *Equal Opportunity Employer.*

FACULTY POSITION
CELL BIOLOGY
University of Texas Southwestern Medical Center at Dallas

The Department of Cell Biology and Neuroscience of The University of Texas Southwestern Medical Center at Dallas seeks applications for a tenure-track **ASSISTANT PROFESSOR** to direct the microscopy and imaging service center for The University. Applicants must have a relevant Ph.D. or M.D. degree, appropriate postdoctoral experience, and be trained in the microscopic sciences. We are particularly interested in creative and highly motivated individuals who have a background using electron microscopy to solve problems in structural biology. The duties of the successful candidate will be to operate a fully equipped imaging facility and to participate in graduate student teaching. Send curriculum vitae, a brief description of proposed research, and three letters of reference to:

Richard G. W. Anderson, Ph.D.
Department of Cell Biology and Neuroscience
UT Southwestern
5323 Harry Hines Boulevard
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OPTICS/RESEARCH FACULTY POSITION

The New England College of Optometry (NEWENCO) is currently seeking applications for a full-time, tenure-track faculty position at the rank of **ASSISTANT** or **ASSOCIATE PROFESSOR.** Responsibilities include teaching geometric and visual optics, conducting research in an optics area relevant to optometry, and obtaining external sources of support. Required qualifications include a Ph.D., a strong research record, and teaching excellence. Salary and faculty rank are negotiable. Applications will be accepted until January 15, 1996.

Applicants should submit a letter of application providing a concise description of research interests and goals, a curriculum vitae, and three professional references to:

James Comerford, Ph.D., O.D.
Chair, Department of Vision Science and Public Health
The New England College of Optometry
424 Beacon Street
Boston, MA 02115

NEWENCO is an Equal Opportunity/Affirmative Action Employer.

ASSISTANT PROFESSOR POSITION IN MICROBIOLOGY
Louisiana State University

Applications are invited from candidates with a background in the use of molecular tools to study the ecology, evolution and/or systematics of prokaryotes. Opportunities are available to interact with LSU's strong interdisciplinary programs in systematics and evolutionary biology. Applicants must have an earned doctorate, postdoctoral research experience, and the ability to develop a strong independent research program. Send curriculum vitae, a statement of research interests, relevant reprints, and the names of three references to: **Search Committee, Department of Microbiology, Louisiana State University, Baton Rouge, LA 70803.** Deadline is November 30, 1995 or until candidate is selected. *Louisiana State University is an Equal Opportunity/Affirmative Action Employer.*

ANIMAL PHYSIOLOGIST

Southern Oregon State College invites animal physiologists to apply for a tenure-track **ASSISTANT PROFESSOR** of Biology position. Duties include teaching animal physiology and participating in a human anatomy-physiology sequence and in introductory biology courses starting September 1996. Ph.D. required. The college focuses on undergraduate education and strongly encourages research involving undergraduates. Send letter of application, curriculum vitae, statement of teaching experience, approach to teaching animal physiology, research plans, list of pertinent courses taken, and names, addresses and telephone numbers of three professional references to: **Dr. Carol Ferguson, Department of Biology, Southern Oregon State College, Ashland, OR 97520. Email: ferguson@wpo.sosc.osshe.edu; FAX: 503-552-6415.** *An Affirmative Action/Equal Opportunity Employer committed to development of an inclusive, multicultural community.*

THE 1996 LOUISA GROSS HORWITZ PRIZE

The Louisa Gross Horwitz Prize was established under the will of the late S. Gross Horwitz through a bequest to Columbia University, and is named to honor the donor's mother. Louisa Gross Horwitz was the daughter of Dr. Samuel David Gross (1805-1889), a prominent surgeon of Philadelphia, and author of the outstanding "Systems of Surgery," who served as president of the American Medical Association.

Each year, since its inception in 1967, Columbia University has awarded the Louisa Gross Horwitz Prize for outstanding basic research in the fields of Biology or Biochemistry. The purpose of this award is to honor a scientific investigator, or group of investigators, whose contributions to knowledge in either of these fields is deemed worthy of special recognition.

The prize consists of an honorarium and a citation which are awarded at a special presentation event. Unless otherwise recommended by the Prize Committee, the prize is awarded annually. The 1995 awardee was Leland H. Hartwell, Ph.D., Professor of Genetics at the University of Washington, in Seattle, WA.

QUALIFICATIONS FOR THE AWARD

The Prize Committee recognizes no geographical limitations. The prize may be awarded to an individual or a group. When the prize is awarded to a group, the honorarium will be divided among the recipients, but each member will receive a citation. Preference will be given to work done in the recent past.

Prospective recipients should be nominated to the Chairman of the Louisa Gross Horwitz Prize Committee, Dr. David I. Hirsh. Nomination letters should include:

1. A summary, preferably less than 500 words, of the research on which this nomination is based.
2. A summary, preferably less than 500 words, of the significance of this research in the fields of biology or biochemistry.
3. A brief biographical sketch of the nominee, including positions held and awards received by the nominee.
4. A listing of up to ten of the nominee's most significant publications relating to the research noted under Item 1.
5. A copy of the nominee's curriculum vitae.

An original and twelve (12) copies of each nomination should be sent to: Dr. David I. Hirsh, Chairman of the Louisa Gross Horwitz Prize Committee, Office of the Vice President for Health Sciences and Dean of the Faculty of Medicine, Columbia University, 630 West 168th Street, New York, New York 10032 .

Deadline for receipt of nominations is January 19, 1996.

POSITIONS OPEN

ASSISTANT PROFESSOR ENVIRONMENTAL SCIENTIST

Illinois Benedictine College is seeking a tenure-track environmental scientist at the **ASSISTANT PROFESSOR** level, to begin August 1996. Ph.D. required. Successful candidate will have terrestrial ecology background and experience. Responsibilities are to teach introductory biology courses and upper-level courses in speciality and to develop a research program involving undergraduates. Cover letter should address applicant's interest in undergraduate teaching in a liberal arts environment. Send curriculum vitae, statement of teaching philosophy and research experience, three letters of recommendation, and all transcripts to: **Dr. Alfred R. Martin, Search Committee, Department of Biology, Illinois Benedictine College, 5700 College Road, Lisle, IL 60532.** Deadline for completed application is December 15, 1995. *Illinois Benedictine College is an Equal Opportunity Employer. Women and minority candidates are especially urged to apply.*

INTEGRATIVE BIOLOGIST

Tenure-track, **ASSISTANT PROFESSOR,** beginning fall 1996. Preference will be given to broadly trained applicants who use modern approaches to study integrative processes at the organismal or population level. Areas of particular interest are evolution, environmental physiology, or behavior. The successful candidate will be expected to establish a vigorous, extramurally funded research program and participate in teaching at the undergraduate and graduate level. Generous set-up funds are available. Applicants should send a statement of research and teaching experience and interests, curriculum vitae, and three letters of recommendation by 15 January 1996, to: **Chair, Integrative Biology Search Committee, Department of Zoology and Genetics, Iowa State University, Ames, IA 50011-3223.** *Iowa State University is an Affirmative Action/Equal Opportunity Employer.*

PROFESSORSHIP ECOLOGICAL/ENVIRONMENTAL ECONOMICS

The University of Minnesota invites applications and nominations for the Interdisciplinary Fesler-Lampert Professorship, an **ENDOWED CHAIR** to be filled in the field of ecological/environmental economics. Applicants should have (1) strong analytical skills in economics that facilitate the application of economic theory and models to significant problems in ecological/environmental economics, (2) a record of substantial research accomplishments reflected in publications in leading journals, (3) experience in and a commitment to incorporating ecological and conservation knowledge into a program of research and teaching that supports a wide range of undergraduate majors, graduate students, faculty and professionals.

The University of Minnesota is a land-grant, research university with strengths in relevant fields such as ecology, economics, natural resources, business management, political science, and international development. The professorship is tenured but rank is open. Salary is competitive. The department(s) in which tenure is held and the teaching and other responsibilities will be negotiated with the successful candidate. Applicants should send a curriculum vitae, a cover letter describing their research, teaching, and service interests, and the names of four references to: **Robert T. Holt, Chair, Fesler-Lampert Search Committee, University of Minnesota Graduate School, 420 Johnston Hall, 101 Pleasant Street S.E., Minneapolis, MN 55455-0421.** *The University of Minnesota is an Equal Opportunity Educator and Employer.*

The Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, is seeking a **PROFESSOR** to develop and administer a basic research program in the molecular biology, pharmacology or genetics of Mood Disorders. The applicant must have a strong scientific and administrative background and be qualified for university appointment at the full professor level. Board eligibility or board certification in general psychiatry is preferred. A tenured appointment at the professor level will be conferred and the appointee may be named to an endowed professorship. Stanford University is committed to increasing representation of women and members of minority groups on its faculty and particularly encourages applications from such candidates. Please submit your curriculum vitae to: **Alan F. Schatzberg, M.D., Chairman of Search Committee, Department of Psychiatry, Stanford University School of Medicine, Stanford, CA 94305-5548.**

POSITIONS OPEN

ASSISTANT PROFESSORS DEPARTMENT OF BIOLOGY Valdosta State University

The Department of Biology is seeking applicants with Ph.D. for the following four tenure-track positions at the level of **ASSISTANT PROFESSOR:** (1) Entomologist; (2) Geneticist, must be able to develop a molecular genetics course; (3) Plant Pathologist, must be able to develop a course in mycology; (4) Aquatic Biologist, must be able to develop a course in toxicology. Research preferences are open for all positions. Appointments are for the fall of 1996. Applicants should submit a letter of application identifying the position for which they are applying, statements of their teaching philosophy and research interests, a curriculum vitae, and three letters of reference by 2 January 1996 to: **Dr. David L. Bechler, Department of Biology, Valdosta State University, Valdosta, GA 31698.** *Valdosta State University is an Equal Opportunity/Affirmative Action Employer.*

THE CLEVELAND CLINIC FOUNDATION

The RESEARCH INSTITUTE of the CLEVELAND CLINIC FOUNDATION

Faculty Position in Inflammatory Bowel Disease Research

We are seeking an individual at the level of **ASSISTANT** or **ASSOCIATE PROFESSOR** to develop a basic laboratory research program in inflammatory bowel disease. The position is jointly sponsored by the Research Institute and the Departments of Gastroenterology and Colorectal Surgery. The successful candidate will possess or exhibit the potential to develop an outstanding national reputation as a basic scientist using cellular and molecular approaches to study the pathogenesis of IBD, including, but not limited to, cytokine and growth factor action, signaling pathways, and immune cell function. This position will be provided with generous startup support. Salary and fringe benefits are highly competitive. The Cleveland Clinic Research Institute has a long standing commitment to excellence in basic and applied biomedical research with an annual research budget in excess of $50 million.

A curriculum vitae and names and addresses of three references shoudl be sent to: **Dr. Thomas A. Hamilton, Department of Immunology, Research Institute, Cleveland Clinic Foundation, NN1, 9500 Euclid Avenue, Cleveland, OH 44195.**

The Cleveland Clinic Foundation is an Equal Opportunity Employer.

BIOMEDICAL SCIENCE

Hampshire College is expanding its program in biomedicine and health science with the appointment of two faculty positions at the Assistant Professor level (Ph.D. required), beginning September 1996, pending funding: 1) **HUMAN BIOLOGIST** working in disciplines such as epidemiology, immunology, reproduction, or endocrinology: 2) **MOLECULAR/CELLULAR BIOLOGIST** working in areas such as immunology, organic toxicology, or molecular genetics.

Hampshire College offers a stimulating and supportive environment for interdisciplinary teaching and collaborative research. We are looking for scientists to help develop our curriculum through the creation of courses combining their research and teaching interests. Courses are designed to engage students in hands-on research. Current faculty have research interests in areas such as women and AIDS, food security in Africa, international health, tourism and health in the Yucatan, undernutrition in local inner city schools, water quality in Sri Lanka, and maternal mortality in Native Americans.

Application review begins on January 2, 1996. Send letter of application, vitae and the names of three referees to the search secretary:

Laurie Smith
Hampshire College
School of Natural Science
Amherst, MA 01002
Email questions to: ljsNS@hamp.hampshire.edu

Hampshire College is an Equal Opportunity Employer and has a vigorous Affirmative Action Program. We especially encourage minorities and women to apply.

POSITIONS OPEN

ASSISTANT PROFESSOR, Life Sciences, two positions: Washington State University: tenure-track, start August 16, 1996. Position1) Ecology; position 2) Physiology/Biochem. Ph.D. in appropriate discipline required by 8/16/96. Ability to conduct innovative, externally funded research program and effectively teach upper-division and graduate courses also required. Substantial, published research, postdoctoral research, and (for position 1) research potentially relevant to the Pacific NW preferred. Position 1 to teach Ecology and the Ecosystem and part of Human Ecology course. Position 2 to teach Mammalian Physiology, Biochemistry, and part of a Biological Methods lab course. Those hired will join the rapidly growing faculty at the new 348 acre Vancouver campus (Portland, Oregon metro area) and help develop the B.S. and M.S. degree programs. Letter of application, vita, statement of research plans, and three letters of reference to: **Dr. Martin Pall, Chair of the Ecology Search Committee or Chair of the Physiology/Biochemistry Search Committee, College of Sciences, Washington State University, Vancouver, WA 98663-3597. Telephone: 360-737-2038; Email: pall@vancouver.wsu.edu.** Screening to begin December 15, 1995. *WSU is an Equal Opportunity/Affirmative Action Educator and Employer. Members of protected groups are encouraged to apply.*

Ecologist/Natural Resource Management—Brigham Young University anticipates filling a tenure-track position at the **ASSISTANT/ASSOCIATE PROFESSOR** level. Position is available September 1, 1996. Position qualifications include Ph.D. or equivalent. Primary responsibilities include teaching and research. Send curriculum vitae, statements of research interests and teaching experience to: **Dr. W. M. Hess, Chair, Department of Botany and Range Science, Brigham Young University, Provo, UT 84602.** *Brigham Young University is an Equal Employment/Affirmative Action Employer. Preference is given to members of the sponsoring church.*

NEUROGERONTOLOGY INSTITUTIONAL PHYSICIAN SCIENTIST PROGRAM

The University of Southern California has recently received an award from the National Institute on Aging for a Physician/Scientist Program for the development of basic and clinical research skills for the study of disease related to the aging nervous system. This is a five-year salary award with appointments in the Department of Neurology of the School of Medicine and in the School of Gerontology. *The candidate must be a U.S. Citizen or have permanent U.S. Resident status* and be board eligible in neurology, psychiatry, neuropathology, or internal medicine. The first two to three years are spent in basic research at the Andrus Gerontology Center or the Hedoo Neuroscience Neural Information and Behavioral Science Program. He or she continues clinical training in neurogerontology and develops an individual research program. Clinical responsibilities are limited to 25% of time and include teaching students, residents, and fellows in neurogerontology throughout the five years. Depending on the background, the candidate could be appointed as **ASSISTANT PROFESSOR** (tenure-track). This position is open immediately. To apply, send a curriculum vitae and three letters of recommendation to: **Leslie P. Weiner, M.D., Department of Neurology, University of Southern California School of Medicine, 1510 San Pablo Street, Suite 646, Los Angeles, CA 90033-4606,** and arrange for two days of interviews. For further information, **Telephone: 213-342-5793.**

PLANT MOLECULAR GENETICIST

The Department of Molecular Genetics and Cell Biology at the University of Chicago is seeking applications for a faculty position at the **ASSISTANT** or **ASSOCIATE PROFESSOR** level in the general field of plant genetics, development and cell biology. The Department seeks an individual who will establish an active research program and participate in both undergraduate and graduate teaching. Particular consideration will be given to highly interactive candidates whose interests complement existing research efforts in pollen-pistil interactions, biochemical genetics, lipid biosynthesis, chloroplast biogenesis and vascular tissue development. Applicants should submit a curriculum vitae, reprints, a summary of current and proposed research work, and should arrange to have three letters of recommendation sent by December 15, 1995 to: **Dr. Anthony P. Mahowald, Department of Molecular Genetics and Cell Biology. The University of Chicago, 920 East 58th Street, Chicago, IL 60637.** *Affirmative Action/Equal Opportunity Employer.*

ARES ADVANCED TECHNOLOGY

THE STRENGTHS OF SERONO

The Ares Serono Group is a pioneering organization widely recognized for turning specific challenges into achievements. At **Ares Advanced Technology**, we employ state-of-the-art methods in Structural Biology, Cell Biology, Molecular Biology and Protein Chemistry to discover and evaluate new biopharmaceutical agents of clinical importance. The exciting, ongoing success of our product portfolio and research projects has triggered the need to add gifted individuals to our staff. Financial stability, innovative products, an entrepreneurial environment and our emergence as a fully integrated company means Ares Advanced Technology can offer you what no one else can-The Best of Biotech.

PRINCIPAL INVESTIGATOR
Protein Purification

In this position, you will take a lead role in the design, execution and optimization of purification methods used to isolate natural and/or recombinant proteins in support of ongoing projects. In addition, you will participate in the evaluation of new proteins as novel therapeutic agents or targets for drug development. Through literature research and attendance at meetings, you will help maintain a state-of-the-art Protein Chemistry Laboratory, making significant contributions to drug discovery and research. Requirements for this position include a Ph.D., a minimum of 4 years' experience, expertise in protein isolation, and familiarity with HPLC and ligand- and immuno-affinity techniques. A knowledge of GLP/GMP guidelines is desirable but not required. Excellent oral and written communications skills are essential. Reference Dept. PC45

Financial Stability

PRINCIPAL INVESTIGATOR
Experimental Therapeutics

We seek an individual with a proven record in the application of immunological techniques to the discovery and development of novel molecules. The responsibilities of this position are to design and implement in vitro and in vivo experimental procedures for the discovery and evaluation of candidate molecules. Minimum requirements include a Ph.D. in Immunology, Cell Biology, or a related field with a minimum of 3 years' post doctoral research experience. The successful candidate will have the ability to work in a multidisciplinary environment, maintain clear and accurate records, and have a strong knowledge of immunology with experience applying these techniques at the cellular and molecular level. Experience with in vivo models of autoimmunity and transplantation is highly desirable. Reference Dept. ET29

Entrepreneurial Environment

SENIOR SCIENTIST
Experimental Therapeutics

Contributing intellectually and technically, you will play a major role in the discovery and evaluation of lead candidates in the areas of oncology, hematology and immunology research. Responsibilities will include the design and implementation of experimental procedures for biologic evaluation of novel compounds such as peptides, small organic molecules and recombinant molecules, as well as in vitro and in vivo assays of immune cell function and hematopoietic cell differentiation. To qualify, you must possess an MS degree and a minimum of 4 years' experience (or a BS degree with a minimum of 6 years' experience) in immunology/hematology or a cell biology related discipline. You should also have a proven track record of technical and theoretical expertise in lymphocyte isolation, cell proliferation assays, flow cytometry and animal modeling. Clear, accurate record keeping is essential, while industrial experience is desired. Reference Dept. ET49

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For consideration, please forward your CV to Ares Advanced Technology, Inc., Human Resources, 280 Pond Street, Randolph, MA 02368. Please reference Department. An Equal Opportunity Employer.

Serono

THE BEST OF BIOTECH

Purdue University

HEAD, DEPARTMENT OF MEDICINAL CHEMISTRY AND MOLECULAR PHARMACOLOGY
School of Pharmacy and Pharmacal Sciences

The Department of Medicinal Chemistry and Molecular Pharmacology, a unique, newly consolidated multidisciplinary unit of twenty-eight faculty with research in the areas of Biochemistry, Bioorganic Chemistry, Medicinal Chemistry, Molecular Pharmacology, Natural Products, Radiopharmaceutical Chemistry, and Toxicology, invites applications and nominations for Department Head.

The preferred candidate will be an outstanding scientist with a distinguished record in research and education. Applicants will be expected to have an established independent research program in at least one of the above-mentioned areas.

The role of the new chair will be to provide scientific, academic and administrative leadership to the Department, as well as to participate in defining the future development of the Department and coordinate the department's interactions with interdisciplinary research programs on the Purdue campus.

Nominations and applications should be sent to:

Professor D.E. Bergstrom, Chair
Department Head Search Committee
Department of Medicinal Chemistry
and Molecular Pharmacology
Purdue University
West Lafayette, IN 47907-1333

Candidates should include in their applications a *Curriculum Vitae*, the names of three references and a letter indicating their interest in the position. Review of applications will begin on December 15, 1995 and continue until the position is filled.

Purdue University is an Equal Opportunity/Affirmative Action Employer and especially encourages applications from interested minority and female candidates.

Faculty Positions in Developmental Biology

Harvard Medical School
Molecular Medicine Unit
Beth Israel Hospital

The Molecular Medicine Unit of Beth Israel Hospital is seeking applications for the position of Assistant Professor. The successful candidate will hold the Ph.D. and/or M.D. degrees, and have completed a minimum of two years of postdoctoral training in molecular cell biology, physiology, or genetics. Applicants will be expected to establish a funded independent research program in the broad area of developmental biology. Generous start-funds and some ongoing support will be provided. The Molecular Medicine Unit faculty currently investigate inductive signaling in early vertebrate development, protein translocation and targetting, signal transduction and ion homeostasis. Candidates should send CVs and names of three references to:

Robert D. Rosenberg, M.D., Ph.D.
Faculty Search Committee
Molecular Medicine Unit
Beth Israel Hospital, RW663
330 Brookline Avenue
Boston, MA 02215

The Beth Israel Hospital is an Equal Opportunity employer.

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Baltimore Convention Center • February 12-13, 1996

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- FREE resume book of all participating candidates

FOR MORE INFORMATION, CONTACT

MAIL: Kevin Bullock
AMSIE'96 Job Fair, 1333 H Street, NW,
Washington, DC 20005

PHONE: (202) 326-7049

FAX: (202) 289-4021

E-mail: kbullock@aaas.org

American Association for the Advancement of Science

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- Bonus for all on-site JOB FAIR registrants: FREE Career Development Seminars on Saturday and Sunday (February 10-11, 1996). Seminars will be conducted by top science recruiters and human resource professionals. Topics include: resume writing, interviewing, and other job-hunting skills, as well as the latest information and issues related to scientific careers
- You can register in advance and just pick up your badge on-site
- You don't have to attend to participate, all resumes will be given to all employers
- FREE to AAAS Members and AMSIE'96 registrants
- FREE pass to the AMSIE'96 Exposition
- Non-member, non-meeting attendees only $25, on-site

FOR MORE INFORMATION, CONTACT

MAIL: Kevin Bullock
AMSIE'96 Job Fair, 1333 H Street, NW,
Washington, DC 20005

FAX: (202) 289-4021

E-mail: kbullock@aaas.org

American Association for the Advancement of Science

AMSIE'96 JOB FAIR

1996 AAAS Annual Meeting & Science Innovation Exposition (AMSIE'96)
Baltimore Convention Center • February 12-13, 1996

Where Science Comes To Life

CANDIDATE Advance Registration Form

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Contact Address _____

City _____ State/Country _____ Zip/Post Code _____

Phone-B (_____) _____ H (_____) _____

EMAIL _____ Visa Type (if non-US citizen) _____

Do you plan to attend AMSIE'96? ❑ Yes ❑ No AAAS Membership No. _____
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DISCIPLINE (please check the <u>one</u> option which best describes your primary discipline)

Agricultural, Biological, & Medical Sciences
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❑ Marine Biology ❑ Medicine ❑ Microbiology ❑ Molecular Biol.
❑ Neuroscience ❑ Pharmacology ❑ Physiology ❑ Zoology

Chemistry
❑ Analytical ❑ Biochemistry ❑ Inorganic ❑ Nuclear
❑ Organic ❑ Physical

Earth Sciences
❑ Atmospheric ❑ Geology/Soil Sci. ❑ Oceanography

Engineering
❑ Aeronautical ❑ Biomedical ❑ Chemical ❑ Electrical/Electronic
❑ Nuclear

Mathematics & Computer Science
❑ Computer Science ❑ Mathematics ❑ Statistics

Physics & Astronomy
❑ Astron./Astrophys. ❑ Atomic/Molecular ❑ Plasma ❑ Nuclear
❑ Optics & Laser ❑ Particle ❑ Condensed Matter

Social & Behavioral Sciences
❑ Anthro./Sociol. ❑ Econ./Poli. Sci. ❑ Education ❑ Psychology
❑ Language Sciences ❑ Science Policy ❑ History & Philosophy of Science

INSTRUCTIONS: Complete the AMSIE'96 Advance Registration Form. Enclose a current curriculum vitae (including a listing of three professional references), and mail to: Kevin M. Bullock, Project Coordinator, AAAS Employment Exchange, 1333 H Street, NW, Suite 1159, Washington, DC 20005. NOTE: Faxed forms and resumes are not acceptable. If you are planning to be on-site at AMSIE'96, please remember to bring multiple copies of your current curriculum vitae. Incomplete forms will not be accepted.

American Association for the Advancement of Science

Research Specialists. The Genetics and Biotechnology Section of Westvaco Forest Research has openings for four research specialists; one each in tissue culture, transformation, sterility and stress resistance of tree species. Responsibilities include assisting in all phases of research including planning, preparation, conducting, evaluation, analysis, and reporting. Qualifications for the tissue culture and transformation positions include a B.S. or M.S. degree in Biological Science, Botany, Forestry, Horticulture or a closely related field. A strong background in plant cell and tissue culture is essential. Experience in plant transformation and genetic engineering is highly desirable. Qualifications for the sterility and stress resistance positions include a B.S. or M.S. degree in Biological Science, Molecular Biology, Biotechnology, or a closely related field. A strong background in plant molecular biology is essential. Experience in nucleic acid isolation, cloning, PCR, and different types of electrophoresis is essential. Preference will be given to candidates with experience in genetic engineering and gene regulation. For all four positions, working experience in woody plants is helpful, but not required. Send resume, college transcripts, and three letters of recommendation before December 15, 1995, to: **Cindy McCord, Westvaco Corporation, P.O. Box 1950, Summerville, SC 29484. For more information contact Cindy McCord at (803) 851-4733, or Fax (803) 875-7185 or E-mail: cmccord@awod.com.**

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DIAGNOCURE INC.

Located in the heart of Québec City, DiagnoCure is a young rapidly growing medical company developing products for cancer diagnosis and treatment. DiagnoCure will employ up to 25 scientists and technologists and has immediate openings for experienced scientists to lead its research unit in the following fields:

Immunodiagnostics: Biotechnology/pharmaceutical experience in development of diagnostics kits. Experience with labeling of monoclonals and fluorescence-based assays would be an advantage.

Recombinant Vaccines: Experience in recombinant vaccine techniques with different vectors, familiarity with anti-idiotypic approaches and synthetic peptides.

Recombinant Peptides: Experience in screening of peptide libraries and reagent development.

Molecular Biology: Experience in characterization of proteins, cDNA cloning and protein structure-function studies.

Each head scientist will report to the Vice President R & D and will play a key role with its scientific advisory committee in determining research strategies. The head scientists will be responsible for the implementation and management of the research programs with the help of support staff. The candidates must have the skills to lead and motivate the staff to perform effectively in a multidisciplinary team under tight schedules.

We offer a highly competitive salary, commensurate with experience, along with the advantages of a progressive, entreprenurial environment.

Please send your c.v. to the:

Vice President of R&D
DiagnoCure Inc.
70, rue Dalhousie, bur. 110
Québec (Québec)
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Committed to quality, diversity and equal employment opportunities.

REGENERON

Postdoctoral/Staff Scientist Positions in Neurobiology

REGENERON is a world leader in elucidating the role of neurotrophic factors in the ontogeny and maintenance of the nervous system and in the development of these factors as therapeutic agents for the treatment of neurodegenerative disorders and neurotrauma. REGENERON has an established reputation for scientific excellence, vigorously encourages publication, and has extensive collaborations with leading academic groups throughout the world. We seek ambitious, career-oriented individuals interested in joining a team involved in cutting edge research.

To augment our multidisciplinary team exploring in vitro and in vivo actions of neurotrophic factors on the peripheral nervous system (PNS), REGENERON has openings for several Ph.D.-level scientists in the Neurobiology Group. We are interested in candidates who have a broad interest in both basic and clinical aspects of the action of neuronal growth factors on the PNS and animal models of sensory and motor neuron dysfunction.

Scientist/Staff Scientist:
Electrophysiology/Behavior
Seeking a Ph.D. with 3 - 6 years postdoctoral training in peripheral nervous system function. Particular emphasis will be given to candidates with the ability to integrate electrophysiological, behavioral or anatomical techniques as they pertain to the normal and pathophysiological function of the PNS.

Postdoctoral Fellow: In vivo studies
Seeking a Ph.D. with 0 - 3 years postdoctoral experience in peripheral nerve studies in vivo. Special emphasis will be given to candidates who show an integrated approach using cellular, molecular and biochemical tools to evaluate the role of neurotrophic factors in the normal maintenance of the PNS and their potential use as treatments for peripheral nerve trauma and neuropathies. Candidates with a specific interest in the system are encouraged to apply.

Postdoctoral Fellow: Cell Biology
Seeking a Ph.D. with 0 - 3 years postdoctoral experience with in vitro approaches to studying peripheral neurons and glial cells. Particular consideration will be given to candidates with an interest in in vitro models of neuron-glia interactions.

Successful candidates for all of these positions will have a proven publication record and demonstrate the ability to integrate into and work well with a project team, and possess good oral and written communication skills. Scientist/Staff Scientist candidates will also have demonstrated ability to initiate independent lines of research.

REGENERON PHARMACEUTICALS, INC. is located in a modern facility in a suburban setting with ready access to the cultural and business centers of New York City. Please send your resume to: **BIN N, Regeneron Pharmaceuticals, Inc., Human Resources Dept., 777 Old Saw Mill River Road, Tarrytown, NY 10591.** *EOE/M/F/H/V*

DGI BIOTECHNOLOGIES (DGI)

DGI is a newly formed biopharmaceutical company focused on developing a novel, small molecule drug discovery platform. This new technology will identify promising drug assays and drug leads for license. Specially designed new laboratory space in Edison, NJ.

MOLECULAR PHARMACOLOGISTS

Experienced in cloning, expressing and asssaying targets such as G-coupled protein, protein hormone, growth and differentiation and transcription factor receptors.

MOLECULAR BIOLOGISTS

Experienced in molecular immunology (including cloning, constructing, expressing and isolating recombinantly derived antibodies). Experience with various protein/peptide phage display system is also desired.

TECHNICAL SUPPORT POSITIONS

For Molecular Biology: strong background in PCR, cloning, digonucleotide based constructions, and auto sequencing.

For Molecular Pharmacology: Receptor isolation, ligand binding with experience with in vitro and in situ reconstruction.

Reply (with Salary Requirements)

Dr. Arthur Blume
DGI BioTechnologies
P.O. Box 424
Edison, NJ 08818-0424
(FAX 908-287-5566)

POSTDOCTORAL RESEARCH POSITIONS

Two to three year positions are available immediately in the molecular genetics laboratory of Richard Straub in the Department of Psychiatry at the Medical College of Virginia. Two projects are funded for the next five years:

Molecular Genetics of Schizophrenia in Irish Pedigrees: project goals are to a) clone the schizophrenia vulnerability locus on chromosome 6, b) identify and confirm additional loci by linkage analysis and other methods and c) clone the additional loci. The primary collaborators are Kenneth Kendler and Charles MacLean.

Detecting Susceptibility Genes for Nicotine Addiction: project goals are to identify genetic loci that influence the susceptibility to cigarette smoking and nicotine dependence. The primary collaborators are Kenneth Kendler, Lindon Eaves and Joanne Meyer.

Applicants should have training and experience in some of the following areas: genotyping, physical mapping , gene identification, mutation detection and DNA sequencing. Experience with the Applied Biosystems DNA sequencer, robotics, and genome informatics is strongly preferred. Salary range is $25,000 and up depending on experience. The Medical College of Virginia offers a generous benefits package, and the laboratory will soon be located in the new Virginia Biotechnology Research Park.

Please send (do not email) curriculum vitae, summary of research experience and interests, and three letters of reference to: **Richard E. Straub, Director, Molecular Genetics Laboratory, Department of Psychiatry, Box 980710, Medical College of Virginia, Richmond, VA 23298-0710.** *Virginia Commonwealth University is an Equal Opportunity/Affirmative Action Employer.*

WYETH-AYERST RESEARCH

Wyeth-Ayerst Research, a major division of American Home Products Corporation, has several opportunities available to work in the areas of Neurobiology, Behavioral Pharmacology and Neuronal Molecular and Cell Biology in the Central Nervous System Research Center located in Princeton, NJ.

Central Nervous System Research

Our goal is to assemble highly motivated multi-disciplinary teams dedicated to the discovery of drugs for the treatment of psychiatric, neurological and neurodegenerative disorders.

Molecular Biologists

B.A./B.S. or M.S. degree with strong background in molecular biology and experience in cell biology and tissue culture techniques, gene cloning and expression, DNA and RNA hybridization, PCR, isotope labeling and mammalian cell transfection. Experience with Western blotting, immunoprecipitation, RNase protection assays and enzymatic assays would be advantageous. **Position #1436**

Neuronal Cell Biologists

B.A./B.S. with experience in cell culture and molecular biology. Candidates will be responsible for maintaining primary and permanent cell lines and will also develop cellular models for CNS disorders. Experience and/or knowledge of the construction of gene expression is required. **Position #1614**

B.A./B.S or M.S. positions to assist with discovery research in neuronal apoptosis, amyloid-related neurodegeneration and growth factor control of neuronal differentiation and survival. These positions will require adaptation and development of culture systems to promote long-term survival of neurons; appropriate support will be provided for high-throughput screening. A working knowledge of neuroanatomy and previous experience in microdissection, neuronal cell culture, cell transfection techniques, fluorescence imaging and cytotoxicity assays is desirable. **Position #1637**

Pharmacologists/Molecular Neuroanatomists/Neurochemists/ Biochemists

Brain Microdialysis: B.A./B.S. or M.S. positions with experience and training in the methodology of brain microdialysis. Working knowledge of modern HPLC techniques with electrochemical and fluorometric detection, familiarity with neuronal histological techniques and small animal surgery. **Position #1581**

Molecular Neuroanatomy – Antibody production: B.A./B.S. positions for production of antibodies for identification, characterization and localization of novel molecular targets. Candidates should have experience with a variety of immunological techniques, including peptide conjugation, ELISA, SDS-PAGE, affinity purification, immunoblotting and immunoprecipitation. Additional experience in cell culture including transient transfection of mammalian cells is highly desirable. **Position #1638**

Candidates with B.S./M.S. degrees and experience in histological processing of brain tissue, brain immunohistochemistry, light microscopy, small animal surgery and behavioral testing for programs in stroke. **Position #1639**

Neuropharmacologist/Senior Biochemical Pharmacologist: B.S./B.A. or M.S. degree with background in one or more of the following areas: receptor binding, second messenger system assays, neurotransmitter release and/or methodology involving the quantification of neurochemical parameters. Experience with cell/tissue culture techniques, small animal surgery, animal-handling and/or in vivo physiological and pharmacological assays, RIAs and/or protein biochemistry is desirable. Candidates with strong backgrounds in enzymology, proteases and protease inhibitors are also invited to apply for these positions. **Position #1611**

B.S./M.S. applicants are sought with skills in immunocytochemistry/in situ living cells in culture. Previous experience with a confocal microscope would be an advantage; knowledge of tissue culture techniques is essential. This position will be responsible for a study of neuro-adaptive changes in the G-protein-mediated signal transducing events underlying psychiatric and neurodegenerative disorders primarily in transfected/mutated cell lines. **Position #1640**

B.S./M.S. individuals [illegible] assays are required for study of regional neuro-adaptive changes in G-protein-coupled receptor-ligand interactions and in signal transduction mechanisms using relevant animal models and transfected/mutated cell lines. Practical experience of protein electrophoresis (1 and 2 dimensional) as well as in vitro second messenger/protein techniques is essential. **Position #1641**

CNS Electrophysiologists

In Vitro Electrophysiologist: Ph.D. with 3 or more years of postdoctoral experience in recording and analyzing specific ion currents from neurons, cell lines, or oocytes using extracellular recording will be considered. Familiarity with generating primary neuronal electrophysiological recordings is essential. **Position #1642**

In Vivo Electrophysiologist: Ph.D. [illegible] electrophysiology and neurobiology. Candidates must be experienced in measuring and analyzing the effects of various psychotropic drugs on electrical activity in specific brain microiontophoretic application of drugs. **Position #1643**

Associate/Senior Pharmacologist: B.A./B.S. with 2-4 years experience or M.S. [illegible] are also required. **Position # 1388**

Behavioral Pharmacologists

Positions available for behavioral pharmacologists with a B.S. or M.S. in neuroscience, psychology, pharmacology, or animal behavior and approximately 1-5 years of [illegible] operant conditioning procedures in rodents, pigeons, and monkeys. Strong analytical, [illegible] is a plus. **Position # 1590**

Wyeth-Ayerst offers an excellent compensation and benefits package in a highly professional environment. Please respond by sending your resume with salary requirements and Position # of interest to: Wyeth-Ayerst Research, Human Resources, P.O. Box 8299, Philadelphia PA 19101. Or you may fax your resume directly into our confidential **Research resume database at (610) 989-4054.** Principals Only. Equal Opportunity Employer, M/F/D/V.

WYETH AYERST

Leading the way for a healthier world.

Faculty Position in Tumor Immunology

Applications are invited to fill **two tenure-track positions** in Tumor Immunology at the Assistant/Associate Professor level in the Department of Pathology, University of Connecticut School of Medicine. Successful candidates are expected to provide leadership to an interdepartmental program in immunology, develop an independent, externally funded research program in the general area of tumor immunology, and participate in the teaching of medical students. To apply, send curriculum vitae, description of research interests, and the names of three references before November 1995 to:

Tumor Immunology Search Committee
Medical Dean's Office
University of Connecticut School of Medicine
263 Farmington Avenue
Farmington, CT 06030-1920

(Search Code 95-186)
An Affirmative Action/Equal Opportunity Employer
M/F/Pwd/V

FRED HUTCHINSON CANCER RESEARCH CENTER

FACULTY POSITION IN CANCER GENETICS

The Fred Hutchinson Cancer Research Center is developing multidisciplinary capabilities to investigate the role of genetics in the etiology of common cancers. We are seeking a molecular geneticist to fill a newly created position at the rank of assistant, associate, or full member (equivalent to assistant, associate, or full professor at a University), with an interest in collaborating with colleagues in conducting population based epidemiologic research. The person hired will be expected to both initiate independent research projects and provide leadership for the development of an active center-wide program that will involve high-volume characterization of known genes in specimens collected from participants in human studies. Resources available include existing population-based studies that involve collection of biological specimens and laboratory facilities. The position can carry a joint appointment at the University of Washington. Send inquiries and curriculum vitae and the names of at least four references by not later than December 15, 1995 to: **David S. Thomas, Program in Epidemiology, Fred Hutchinson Cancer Research Center, 1124 Columbia Street, WA, 98104.** *The Fred Hutchinson Cancer Research Center and the University of Washington are equal opportunity/affirmative action employers. Both institutions are building culturally diverse faculty and strongly encourage applications from female and minority candidates.*

The deadline for applications is December 15, 1995.

ACADEMIC EXCELLENCE GEORGIA SOUTHERN UNIVERSITY

The Department of Biology; Georgia Southern University seeks applications for two tenure track openings at the level of Assistant Professor to begin September 1, 1996. Salary is competitive and commensurate with experience and qualifications. Candidates for both positions must have received a Ph.D. before January 1996. Postmark deadline for both positions is January 2, 1996.

Systematic Biologist utilizing-modern molecular techniques as a primary research tool. Teaching responsibilties may include participation in introductory biology and one or more of the following specialty courses: molecular biology, biometry, genetics, systematics, and cell biology. Applicants should send a letter of application, curriculum vita, a statement of teaching and research interests, and the names of three references to: **Dr. Denson K. McLain, Systematics Search, Department of Biology, Landrum Box 8042, Georgia Southern University. Statesboro, GA 30460-8042, USA.**

Physiological Ecologist / Aquatic Biologist able to work in the wetlands or estuaries of the Southeastern Coastal Plain. Teaching responsibilities may include participation in introductory biology and one or more of the following courses: Physiology, Human Anatomy and Physiology, and upper level courses in the area of specialty. Applicants should send a letter of application, curriculum vita, a statement of teaching and research interests, and the names of three references to: **Dr. John W. Parrish, Physiology Search, Department of Biology, Landrum Box 8042, Georgia Southern University. Statesboro, GA 30460-8042, USA.**

The names of applicants and nominees, resumes and other general non-evaluative information are subject to public inspection under the Georgia Open Records Act. Georgia Southern University is an Equal Opportunity/Affirmative Action Institution. Persons who need accomodations(s) in the application process under the *Americans with Disabilities Act* should notify Dr. John Averett, Department Chair.

Dean of Natural Sciences and Mathematics
California State University, Long Beach

The College of Natural Sciences and Mathematics, consisting of the Departments of Biological Sciences, Chemistry and Biochemistry, Geological Sciences, Mathematics, Physics and Astronomy, and Science Education, are recruiting for the position of Dean. The University enrollment approximates 27,000; there are about 1,500 majors in the College served by 175 faculty. All departments in the College, except for Science Education offer the bachelor's and master's degrees. The successful candidate must possess an earned doctorate and be eligible for appointment as a tenured faculty member in one of the College's departments. The candidate must be committed to research and have a strong publication record, have had College or University teaching experience, be open to faculty governance and consultation, have successful academic administrative experience, have been involved in fiscal management and fund raising efforts, and have the ability to communicate effectively with an ethnically and culturally diverse campus community. Review of applications will begin on January 9, 1996; the position will remain open until filled. Preferred starting date is July 1, 1996. Applications must include a resume, a letter of interest that addresses qualifications, and a list of at least three professional references including names, addresses, and telephone numbers. Nominations are also invited. Send applications, nominations, and requests for more detailed information about the position to **Dr. Kenneth Marsi, Chair, Dean Search Committee, Office of the Vice President for Academic Affairs, California State University, Long Beach, 1250 Bellflower Blvd., Long Beach, CA 90840.** *California State University, Long Beach is an Equal Opportunity/ Affirmative Action, Title IX Employer, and is in compliance with the Civil Rights Act of 1964 Title VI and Title VII), Title IX of the Education Amendments of 1972, the Rehabilitation Act of 1973, the Age Discrimination Act of 1975, and the Americans with Disabilities Act of 1990.*

Grand Valley State University, a regional comprehensive university which emphasizes excellence in teaching, outreach endeavors within its geographic region, and faculty/student research collaboration, is seeking a chair for the Biology Department of 15 full time faculty. This is a tenure track position at the Associate or Professor rank. Qualifications include a Ph.D. in biology or related field as well as demonstrated success in teaching, academic leadership, and research. The successful candidate will have demonstrated experience in managing budgets, developing curricula, nurturing faculty development, and providing opportunities for scholarly activity.

The Biology Department is a dynamic unit offering degrees in biology and natural resources management to approximately 400 majors. This department is in a growth phase, in the process of moving into a newly constructed science facility, considering new curricular options, and adding new faculty positions.

Grand Valley State University, the fastest growing educational institution in Michigan, is a fully accredited, state supported, four year and graduate degree granting institution with a current enrollment of nearly 14,000 students. The main campus comprises 900 acres conveniently located near the city of Grand Rapids and the shores of Lake Michigan. This region of west Michigan offers diverse cultural and leisure activities, and prides itself on a high quality of life enhanced by a moderate cost of living.

Applicants should submit a letter of interest describing how their qualifications meet the position requirements, a statement that describes their academic and administrative philosophy, a curriculum vita and name/addresses/phone numbers of three references to:

P. Douglas Kindschi, Dean of Science and Mathematics
312 Padnos Hall of Science
Grand Valley State University
Allendale Michigan 49401

The position is available starting Fall 1996. Consideration of completed applications will begin on November 27, 1995, and the position will remain open until it is filled. *EEO/AA/ADA*

TOXICOLOGIST

Midwest Research Institute (MRI), a leading research organization, is seeking an experienced toxicology professional to join our Life Sciences Department.

This individual will conduct animal toxicology studies, prepare and review protocols and reports, monitor financial performance of projects, supervise staff, evaluate training needs of staff and arrange for appropriate training. Schedule personnel and facilities, contribute to scientific literature, develop new methods and technologies as appropriate to new markets, interact and communicate with clients. Requires a Ph.D. in pharmacology, toxicology or related discipline plus a minimum of three years experience in toxicology studies with a minimum of one year as a study director. Supervisory and project management experience a must. Excellent understanding of GLP requirements, contract research, business development and client orientation essential. DABT desirable.

MRI offers competitive compensation and a comprehensive benefits package and a unique environment to meet personal challenges and enhance career growth. If you are interested in being a part of this select group of professionals, please send your resume marked 95-342-3 to: Midwest Research Institute, 425 Volker Boulevard, Kansas City, MO 64110-2299, or fax it to (816) 753-2304, EEO/AA, M/F/D/V. A Drug Screening Employer

DIRECTOR

BELLE W. BARUCH INSTITUTE FOR MARINE BIOLOGY & COASTAL RESEARCH

THE UNIVERSITY OF SOUTH CAROLINA invites nominations and applications for the position of Director of the Baruch Institute, a major research facility at the University of South Carolina. The Search Committee will begin reviewing applications on January 8, 1996.

The Baruch Institute for Marine Biology & Coastal Research is a free-standing unit of the College of Science and Mathematics that provides facilities and resources for faculty and student research. Since the Baruch Institute was founded in 1969, associates have attracted $24.4 million in external funding; this year's funding is $4.1 million. Over 1050 scientific articles covering a variety of disciplines have been published, and 250 theses have been generated from student research. There are currently about 60 graduate students and 70 associated faculty conducting research and providing public education on basic and applied problems related to the marine sciences. Utilizing a multidisciplinary approach, the Institute participates in a wide range of studies that involve many departments, institutions, and government agencies. Current projects encompass molecular biology, geochemistry, ecotoxicology, sedimentology, spatial and coastal processes, watershed hydrology, and ecology.

The Institute is housed at the main campus of the University of South Carolina in Columbia and operates the Baruch Marine Field Laboratory on the 17,500 acre Hobcaw Barony near Georgetown on the South Carolina coast. Additional facilities under the Institute's purview are located in Charleston Harbor and at Pritchard's Island near Beaufort.

The Director will be an accomplished, respected scientist with demonstrated excellence in marine research, administration, and communication, as well as entrepreneurial abilities. The successful candidate will be appointed at the rank of Professor in an appropriate academic department and in the Marine Science Program. The Director reports to the Dean of the College of Science and Mathematics.

Letters of Nomination should include the nominee's name, current position, address, tel/fax number(s), and e-mail address. **Applications** should include a letter of interest, a summary of the applicant's qualifications, a curriculum vitae, and the names of five individuals who have been asked to supply references (include each referee's address, tel/fax number(s), and e-mail address). Nominations and applications should be sent to:

Prof. Bruce C. Coull
Chair of Baruch Institute Search Committee
College of Science and Mathematics
University of South Carolina
Columbia SC 29208
Tel: 803-777-3940 • Fax: 803-777-9385 • E-mail: bccoull@sc.edu

The University of South Carolina is an Equal Opportunity/Affirmative Action Employer.

Faculty Positions in Neurobiology

University of Maryland School of Medicine
Department of Anatomy

The Department of Anatomy is undergoing major expansion and rebuilding. New faculty are being recruited in neurobiology. Positions are available at all ranks.

The Department is located in a newly opened building. Significant expansion of research resources is underway, including core laboratories in molecular biology, tissue culture, neuroanatomy, imaging, and neurophysiology.

Successful candidates will have a Ph.D. or equivalent, outstanding ability in teaching, and exceptional achievement in their field of research, including the potential to attract external funding. Investigators in the Department use approaches ranging from molecular biology to behavior but the common focus is understanding the *organization*, *function* and *development* of neural networks. We particularly encourage applicants investigating the:

- interplay of gene expression and neural activity in neural network development.
- neurobiological bases of learning and memory.
- development and functional organization of chemosensory systems.

The Department teaches a 9.5 week course - "Structure & Development" - in the medical school curriculum. New faculty are expected to participate 3-5 weeks in this team taught course. Graduate teaching is also encouraged.

For best consideration, applications should be received before February 1, 1996. Candidates should send a C.V., summary of research accomplishments and goals, and the names of 3-5 individuals from whom recommendations may be obtained to:

Dr. David V. Smith
Chair, Faculty Search Committee
Department of Anatomy
University of Maryland School of Medicine
655 West Baltimore Street
Baltimore, MD 21201-1559

The Department is eager to diversify its faculty. We encourage minorities and women to apply. The University of Maryland is an AA/EEO/ADA employer.

Executive Dean of Agriculture and Natural Resources

Executive Director of the New Jersey Agricultural Experiment Station
and
Dean of Cook College

Rutgers, The State University of New Jersey seeks candidates for Executive Dean of Agriculture and Natural Resources with responsibilities for George H. Cook College and the New Jersey Agricultural Experiment Station (NJAES). Cook College serves over 3,500 students, offering undergraduate and graduate professional programs across seventeen academic departments. The Agricultural Experiment Station encompasses sixteen research centers and field stations and maintains a network of outreach and service, including Rutgers Cooperative Extension (RCE).

Together, Cook and NJAES epitomize the modern land-grant college and experiment station. The mission is to educate students and to address and resolve the social, economic, physical, biological, and policy dimensions of contemporary and future issues in agriculture and natural resource areas. These include agricultural production and competitiveness, food science and engineering; nutrition, health, and safety; marine and coastal resources; natural resources and environment; and human and community resources and development. The annual appropriated budget is $40 million and there are approximately 300 full-time faculty.

As executive dean and director, the individual reports for NJAES to the president of the university, and for Cook College to the provost of the New Brunswick Campus. The successful candidate should be an established scholar of international stature. In addition, evidence of administrative achievement; excellence in research and teaching; experience in outreach, extension, and service and demonstrated leadership ability at both the governmental and university level are expected.

Applications and nominations will be accepted until **December 15, 1995**, or until a suitable candidate is found. Letters of application or nomination, including a current vita, should be sent in confidence to:

THE STATE UNIVERSITY OF NEW JERSEY
RUTGERS

Dr. Harry Janes
Chair, Search Committee
c/o Office of the Provost and Graduate Dean
Rutgers, The State University of New Jersey
18 Bishop Place
New Brunswick, NJ 08903

Employment eligibility verification is required
An Affirmative Action/Equal Opportunity Employer

MICHIGAN STATE UNIVERSITY
Department of Biochemistry

The Department of Biochemistry seeks applications for a tenure-track position at the **ASSISTANT PROFESSOR** level. Applicants should have demonstrated productivity and evidence of potential for independent research on aspects of the biochemistry of the cell nucleus. We are particularly interested in candidates with research programs in nuclear structure, nuclear transport, nuclear signaling, regulation of transcription, DNA replication or RNA processing. The successful applicant will be expected to participate in the Program for Studies on the Biochemistry of the Cell Nucleus, an area of emphasis in the Biochemistry Department developed with support from the Michigan State University Cancer Center. The Biochemistry faculty has explicitly endorsed efforts to increase the diversity of its ranks, and accordingly, candidates from groups currently underrepresented in academic science are encouraged to apply.

The Department of Biochemistry has a large faculty conducting research in most areas of contemporary biochemistry and molecular biology, providing both a stimulating scientific atmosphere and opportunities for research collaboration. The Department occupies a modern research building with well-equipped research and teaching laboratories, and has vigorous undergraduate, graduate, and postdoctoral training programs.

Applicants should submit a curriculum vitae, a description of research accomplishments and future interest, and have three letters of recommendation sent on their behalf to: **Dr. William L. Smith, Chairperson, Department of Biochemistry, Box S, Michigan State University, East Lansing, MI 48824-1319.** To ensure consideration, applications should be received by December 1, 1995. *Michigan State University is an Affirmative Action/Equal Opportunity Employer. Handicappers have the right to request and receive reasonable accommodation.*

POSTDOCTORAL POSITION
MASSACHUSETTS GENERAL HOSPITAL
Harvard Medical School
Cutaneous Biology Research Center

A POSTDOCTORAL RESEARCH POSITION is available immediately to study mechanisms of TGF-β signaling during *Drosophila* development. Work involves molecular genetic approaches to study novel proteins required for maximum *decapentaplegic* function. Our goal is to elaborate the molecular pathways regulating *dpp* signaling and understand how this signal can elicit different developmental events. The position requires a Ph.D. and experience in molecular techniques. Salary is negotiable according to experience and institutional guidelines. Send curriculum vitae, one publication, and three letters of reference to:

Laurel Raftery, Ph.D.
Massachusetts General Hospital
Cutaneous Biology Research Center (CBRC)
Building 149, 13th Street
Charlestown, MA 02129

The Massachusetts General Hospital/Harvard Cutaneous Biology Research Center is a committed Equal Opportunity/Affirmative Action Employer. Minorities, women, handicapped and veterans are encouraged to apply.

POSTDOCTORAL POSITION
VASCULAR AND MOLECULAR BIOLOGY

We are pursuing the cellular and molecular mechanisms by which endothelium-derived nitric oxide regulates oxidant-responsive transcriptional pathways mediating endothelial-monocyte interactions. Our lab is vertically integrated, with molecular, cellular, whole animal, and human research capabilities. Background in cell/mol/biol and *U.S. citizenship required*. Send curriculum vitae and reprints to: **John P. Cooke, M.D., Ph.D., Division of Cardiology, Stanford University School of Medicine, 300 Pasteur Drive, Stanford, CA 94305-5246.**

HARVARD UNIVERSITY
Postdoctoral Position

Applications are invited for a **POSTDOCTORAL POSITION** to study intracellular transduction of the insulin signal. Candidates should have a recent Ph.D. or Ph.D/M.D. with experience in biochemistry or molecular biology. Please send curriculum vitae and the names, addresses and telephone numbers of three references to: **Dr. David Sacks, BWH, Thorn 430, 75 Francis Street, Boston, MA 02115.** *U.S. citizenship or permanent residency required.*

FACULTY POSITIONS
DEPARTMENT OF MICROBIOLOGY
NEW YORK UNIVERSITY
SCHOOL OF MEDICINE

The Department of Microbiology of NYU School of Medicine is seeking to fill one to two tenure-track positions at the **ASSISTANT/ASSOCIATE PROFESSOR** level within the next one to two years. We are particularly interested in attracting outstanding, researchers with a Ph.D. or M.D. degree working in the fields of viral and bacterial pathogenesis but will also welcome applications from individuals interested in the molecular analysis of viral and bacterial function in general. Participation in the Department's graduate teaching is expected. Start-up funds are available.

The Department of Microbiology is housed in the Medical Science Building at NYU Medical Center, with additional faculty located in the adjacent Skirball Institute of Biomedical Research and nearby Aaron Diamond AIDS Research Center and Public Health Research Institute. This research interests of the faculty are diverse with an emphasis on mechanisms of microbial pathogenesis including the study of AIDS and retrovirology, host defense mechanisms, microbial and molecular genetics, oncogenesis, growth factors, cytokines and the regulation of gene expression. The Department has an active NIH-supported graduate training program and also participates in interdisciplinary NIH-funded programs in the Kaplan Cancer Center and the Center for AIDS Research. Applicants should send their curriculum vitae, names of three references and a brief statement of research interests to: **Microbiology Search Committee, Department of Microbiology, NYU Medical Center, 550 First Avenue, New York, NY 10016.** *NYU is an Equal Opportunity Employer.*

ASSISTANT PROFESSORSHIPS
BIOLOGY

The Department of Biology at West Virginia University invites applications for two tenure-track positions at the level of beginning **ASSISTANT PROFESSOR** in the area of Cellular and Molecular Biology. Subject to funding approval, these positions will be available August 15, 1996. Applicants must have a Ph.D., or equivalent, with appropriate postdoctoral training. Teaching is an important consideration and the successful candidates will be expected to teach in the undergraduate and graduate programs. Each candidate will be required to establish a strong externally funded research program. We are seeking scientists whose research interests are in the bioregulation of aging, bone, *Drosophila* genetics, or reproduction, but strong candidates in other areas will be considered. Please submit a curriculum vitae, a statement of teaching and research interests, and provide the names, addresses, and telephone numbers of three referees to: **Cellular and Molecular Biology Search Committee, P.O. Box 6057, Department of Biology, West Virginia University, Morgantown, WV 26506-6057.** Review of applications will begin on December 15, 1995. *West Virginia University is an Equal Opportunity Employer. Women, individuals with disabilities and minority candidates are encouraged to apply.*

POSTDOCTORAL POSITION available immediately for NIH-funded research that is directed at defining the molecular target(s) of alcohol and volatile general anesthetics. Our approach is to use gene targeting in embryonic stem cells to create novel mouse lines that harbor modifications in genes putatively identified as critical to the physiologic response to ethanol and/or anesthetics. Prior experience in molecular biology, transgenic animal technology, and/or pharmacology is preferred but not required. Send letter of interest and curriculum vitae to: **Gregg E. Homanics, Ph.D., University of Pittsburgh, Department of Anesthesiology, W1356 Biomedical Science Tower, Pittsburgh, PA 15261.** *The University of Pittsburgh is an Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL FELLOW/RESEARCH ASSISTANT/ASSOCIATE to work on the molecular regulation of nitric oxide-guanylyl cyclase signalling pathway in the context of vascular physiology and pathophysiology with dynamic vascular biology research group at the University of Virginia. Molecular biology expertise essential, promoter analysis experience and cardiovascular background preferable. Applications detailing molecular biology experience and names of three references to: **Roger A. Johns, M.D., University of Virginia Health Sciences Center, Box 238, Charlottesville, VA 22908 U.S.A.** *An Equal Opportunity/Affirmative Action Employer.*

MICHIGAN STATE UNIVERSITY
Department of Biochemistry
National Food Safety and Toxicology Center

The Department of Biochemistry in conjunction with the National Food Safety and Toxicology Center (NFSTC) at Michigan State University seeks applicants for a tenure-track position at the **ASSISTANT PROFESSOR** level in biochemical toxicology. Applicants should have postdoctoral experience demonstrating unusual productivity and further potential to develop a strong externally-funded research program in the toxicological aspects of gene regulation and the biochemistry of the cell nucleus. The research focus should be directed to contemporary problems of food-borne toxicants. The motivation to act as a team member in collaborative and interdisciplinary research groups will be an important characteristic of the successful candidate.

The Department of Biochemistry has a large faculty conducting research in most areas of contemporary biochemistry and molecular biology. It occupies a modern research building with well-equipped research and teaching facilities and vigorous undergraduate, graduate, and postdoctoral training programs. The NFSTC expects to open its new laboratory building nearby in 1997. It will provide an outstanding environment for food toxicology research with many possibilities for collaboration with other scientists in toxicology, pathology, epidemiology and the food and agricultural sciences.

This is primarily a research position with some additional teaching responsibilities. It is expected that through graduate teaching activities, the successful candidate will contribute to the campus-wide program in Environmental Toxicology.

Applications should submit a curriculum vitae, a description of research accomplishments and future interests, and have three letters of recommendation sent to: **Dr. William L. Smith, Chairperson, Department of Biochemistry, Box ST, Michigan State University, East Lansing, MI 48824-1319.** Review of applications will begin December 1, 1995 and will continue until a suitable candidate is found. *Michigan State University is an Affirmative Action/Equal Opportunity Employer. Handicappers have the right to request and receive reasonable accommodation.*

NMR SPECTROSCOPY

The Cardiovascular NMR Section at Columbia University seeks applicants for the position of **POSTDOCTORAL RESEARCH SCIENTIST** with an M.D. or Ph.D. degree and one to two years of postdoctoral experience in NMR spectroscopy, with interest in becoming involved in studies of perfused hearts and isolated cardiac myocytes. Expertise with NMR studies of cell systems and with multiple-quantum NMR is especially desirable. Our current interests are in pursuing multinuclear spectroscopy including proton, sodium, and phosphorus. In addition, the candidate will have the opportunity to become involved in multinuclear MR imaging. This is primarily a research position with no clinical responsibilities. Please send curriculum vitae, a reprint of a key publication, and the names of two references to: **Dr. Jose Katz, Division of Cardiology, College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York, NY 10032.** *Columbia University is an Affirmative Action/Equal Opportunity Employer.*

Two **POSTDOCTORAL POSITIONS,** available immediately, to study an adhesive multicomponent system expressed by an enterobacterial pathogen. Requires experience in 1) molecular genetics (bacterial genetics, recombinant DNA, mutagenesis, PCR) or 2) glyco-protein/lipid biochemistry and use of antibody probes. Send curriculum vitae, description of previous experience and references to: **Dr. Dieter M. Schifferli, University of Pennsylvania, Department of Pathobiology, 3800 Spruce Street, Philadelphia, PA 19104-6049. FAX 215-898-7887.** *Affirmative Action/Equal Opportunity Employer.*

Two **POSTDOCTORAL POSITIONS,** available immediately, to study an adhesive multicomponent system expressed by an enterobacterial pathogen. Requires experience in 1) molecular genetics (bacterial genetics, recombinant DNA, mutagenesis, PCR) or 2) glyco-protein/lipid biochemistry and use of antibody probes. Send curriculum vitae, description of previous experience and references to: **Dr. Dieter M. Schifferli, University of Pennsylvania, Department of Pathobiology, 3800 Spruce Street, Philadelphia, PA 19104-6049; FAX 215-898-7887.** *Affirmative Action/Equal Opportunity Employer.*

The University of Tennessee, Knoxville

DEAN

College of Arts & Sciences

The University of Tennessee, Knoxville invites applications and nominations for Dean of the College of Arts and Sciences. The Dean is the chief administrative officer of the College, reporting directly to the Vice Chancellor for Academic Affairs. The Dean is responsible for leading the largest of the university's academic divisions. The College includes 27 academic departments and 13 interdisciplinary programs and offers undergraduate majors and graduate programs of study spanning the arts, humanities, natural sciences, and social sciences.

We seek candidates whose personal and professional qualities and experience assure energetic and creative leadership in all areas of academic endeavor. Candidates must have: (1) significant academic and administrative experience and merit appointment as a full professor with tenure in one of the disciplines represented in the College; (2) experience in planning, budgeting, and fund raising; (3) a belief in faculty governance; (4) an awareness of national issues surrounding university education and a vision for addressing them; (5) a record of commitment to diversity in the faculty and student body; and (6) a commitment to excellence in teaching, research, and service at a major research and/or land-grant university.

We seek to fill this position by July 1, 1996. The University especially welcomes as candidates persons from traditionally underrepresented groups. We will begin screening applications immediately and will consider all applications and nominations until the position is filled. Salary is competitive. Send nominations or a letter of application (including a current curriculum vitae and names, addresses, and phone numbers of four references) to: Dr. Linda Maxson, Chair
Arts & Sciences Dean Search
The University of Tennessee
1819 Andy Holt Ave.
Knoxville, TN 37996-4350

UTK is an EEO/AA/Title IX/Section 504/ADA Employer.

SWARTHMORE

The Department of Biology at Swarthmore College invites applications for three different one-year positions at the assistant professor level, beginning September 1996. Applicants should have a Ph.D., teaching experience and a strong commitment to undergraduate education. Swarthmore College is an equal opportunity employer.

Plant Physiology. Teaching duties will include a one semester course in plant physiology with laboratory, an advanced course/seminar with laboratory in the area of the applicant's special interest, and participation in a team-taught introductory biology course and a team-taught senior seminar. Interested persons should submit a curriculum vitae, three letters of recommendation and a statement of teaching and research interests to: **Plant Physiology Search, Department of Biology, Swarthmore College, Swarthmore, PA 19081.** All materials must be received by **January 5, 1996.**

Neurobiology. Experience with neurophysiological recording techniques is required. Teaching duties will include a course in cellular neurobiology with laboratory, an advanced course/seminar in some area of neuroscience with lab projects, and participation in a team-taught introductory biology course and a team-taught senior seminar. Interested persons should submit a curriculum vitae, three letters of recommendation and a statment of teaching and reserach interests to: **Neurobiology Search, Deparment of Biology, Swarthmore College, Swarthmore, PA 19081.** All materials must be received by **January 10, 1996.**

Animal Behavior. Recent research experience is required and should include field work, preferably with insect or primate social systems. Teaching duties will include participation in a team-taught introductory biology course, a one semester course in general animal behavior with laboratory, an advanced course/seminar with laboratory projects in the area of the applicant's interest, and participation in a team-taught senior seminar. Interested persons should submit a curriculum vitae, three letters of recommendation and a statement of teaching and research interests to: **Animal Behavior Search, Department of Biology, Swarthmore College, Swarthmore, PA 19081.** All materials must be received by **January 19, 1996.**

The University of ILLINOIS at Urbana-Champaign

ASSISTANT PROFESSOR
Microbiology

The Department of Microbiology at the University of Illinois at Urbana-Champaign seeks candidates for a full-time tenure-track faculty position at the Assistant Professor level. Applicants should have a Ph.D. degree, postdoctoral experience, and show exceptional promise in developing an active research program in Microbiology. Preference will be given to applicants with research interests in the physiology, biochemistry, or molecular genetics of microorganisms that further expand the Department's breadth of microbial diversity. Responsibilities include teaching undergraduate and graduate students. Starting date August 1996. Salary will be commensurate with experience.

The Department of Microbiology offers a supportive and collegial atmosphere and a strong graduate program. There are extensive interdepartmental interactions in microbial and eukaryotic cell and molecular biology, immunology, developmental biology, evolution, biochemistry, and biophysics. The University of Illinois at Urbana-Champaign offers an outstanding and exciting academic research environment including a Biotechnology Center and state-of-the-art facilities for hybridoma production, flow cytometry, electron microscopy, X-ray crystallography and computer analyses.

Applicants should submit a curriculum vitae, a description of research interests and future plans and have at least three letters of reference sent to:

Abigail Salyers, Chair
Faculty Search Committee
Department of Microbiology
University of Illinois
131 Burrill Hall
407 S. Goodwin Avenue
Urbana, IL 61801
Telephone (217) 333-1736
FAX (217) 244-6697

To ensure full consideration, applications should be received by December 15, 1995. Interviews may be conducted before the closing date, but no final decision will be made before that time.

The University of Illinois is an Affirmative Action-Equal Opportunity Employer.

TENURE-TRACK FACULTY POSITIONS AT CARB

PROTEIN STRUCTURE AND FUNCTION

The Center for Advanced Research in Biotechnology (CARB) is seeking to fill two tenure-track faculty positions in (1) the elucidation of molecular mechanisms in enzyme catalysis and (2) receptor-mediated signal transduction. Applicants using multidisciplinary approaches in biochemistry, structural and molecular biology are invited to apply. The successful candidates will be expected to develop strong, independent, externally funded research programs, and also to interact with other CARB scientists involved in physical biochemistry, structural immunology, protein folding, stability, modeling and engineering, macromolecular crystallography, computational chemistry and high-field NMR spectroscopy. One position will be a joint appointment with Bowie State University of the University of Maryland System.

CARB is a research center of the National Institute of Standards and Technology (NIST) and the University of the Maryland Biotechnology Institute devoted to fundamental problems of macromolecular structure, function and engineering. Interested candidates should send a curriculum vitae, three letters of reference, and a summary of research interests and plans to:

Faculty Search Committee
Center for Advanced Research in Biotechnology
University of Maryland Shady Grove
9600 Gudelsky Drive
Rockville, MD 20850
USA

Review of applications will begin on **January 15, 1996.** *CARB is an Equal Opportunity/Affirmative Action Employer. Women and minority candidates are encouraged to apply. For one of the positions, preference will be given to applicants who are U.S. citizens.*

POSITIONS OPEN

POSTDOCTORAL POSITION REPRODUCTIVE BIOLOGIST/EMBRYOLOGIST

A **POSTDOCTORAL POSITION** is available for a period of at least two years to study cell cycle regulation, cytoplasmic and nuclear transfer in ascidian, mammalian and human oocytes. The applicant will preferably have experience in biochemical and molecular assays of cell cycle regulation and/or micromanipulation. The position will be supported by The Center of Reproductive Medicine in Englewood, Colorado and The Institute for Reproductive Medicine and Science at Saint Barnabas Medical Center, Livingston, New Jersey, under the joint supervision of **Drs. Jacques Cohen, Steen Willadsen** (St. Barnabas), and **Brian Dale** (Stazione Zoologica, Napoli). It is expected that most of the experimental work will be carried out at Saint Barnabas Medical Center. To apply send résumé and two reference letters to: **Jacques Cohen, Scientific Director of Assisted Reproduction, The Institute for Reproductive Medicine and Science at Saint Barnabas, 101 Old Short Hills Road, Suite 501, West Orange (Livingston), NJ 07052.**

POSTDOCTORAL POSITION funded by NCI through April 1998, available January 1, 1995, to perform structure/function studies on *qph*, a novel oncogene isolated from neoplastic Syrian hamster embryo cells (*Oncogene* 9:2065-2069, 1994, and 10:963-971, 1995). Primary research includes: a) isolation and characterization of the promoter region and upstream sequences of the hamster *qph* oncogene and proto-oncogene; b) modulation of *qph* expression as part of the cellular response to stress; c) structural characterization of the human *qph* proto-oncogene and cDNA; d) identification of its protein product, and e) detection for *qph* alterations in human primary tumors and tumor-derived cell lines. Experience with molecular biology techniques (cloning, sequencing, PCR, protein electrophoresis, immunodetection, and purification) is required. Interested individuals should send curriculum vitae and names of three references to: **Dr. V. Notario, Experimental Carcinogenesis Program, Department of Radiation Medicine, Georgetown University Medical Center, TRB E220A, 3970 Reservoir Road, NW, Washington, DC 20007.** *Georgetown University is an Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL POSITION available to study association/folding processes between protein fragments using multidimensional NMR spectroscopy (*Tasayco* and **Chao**, *Proteins*, 22:41–44 1995). Experience solving the structure of proteins in solution is essential. Send curriculum vitae including list of publications, names and addresses of three references to: **Dr. Maria Luisa Tasayco, Chemistry Department, City College of the CUNY, Convent Avenue and 138th Street, New York, NY 10031. Email: mltj@carmen.sci.ccny.cuny.edu.**

POSTDOCTORAL POSITIONS available immediately to study mechanisms of growth factor and oncogene intracellular signaling and to develop new cancer drugs based on the modulation of these signaling pathways. Applicants must have a Ph.D., M.D., or equivalent degree and a background in biochemistry, molecular biology, cell biology, or pharmacology. The position offers the opportunity for independent research in a well-equipped multidisciplinary lab in an attractive work environment. Applications with curriculum vitae and three letters of recommendation should be sent to: **Dr. Garth Powis, Arizona Cancer Center, University of Arizona Health Sciences Center, 1515 North Campbell Avenue, Tucson, AZ 85724-5024.** Application review begins November 10, 1995 and continues until December 15, 1995. *The University of Arizona is an Equal Employment Opportunity/Affirmative Action/Americans with Disabilities Act Employer.*

A SENIOR RESEARCH ASSOCIATE POSITION and a **POSTDOCTORAL POSITION** available immediately to study molecular mechanism of tumorigenesis, involving tumor suppressor genes and oncogenes. Very competitive salary depending on experience. Please send curriculum vitae with names and telephone numbers of three references to: **Yuen Kai Fung, Ph.D., Associate Professor, University of Southern California, Childrens Hospital Los Angeles, 4650 Sunset Boulevard, Mailstop 94, Los Angeles, CA 90027.** *Equal Opportunity Employer.*

Agricultural Research Service — United States Department of Agriculture

POSTDOCTORAL RESEARCH ASSOCIATE POSITION: The U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Sugarbeet and Bean Research Unit, East Lansing, Michigan, is seeking an individual to isolate and characterize dry bean seed coat pigments (flavonoid compounds) and to conduct research that relates Mendelian genes conditioning seeds coat pigments to multistep biosynthetic pathways by which the pigments are formed and interact. Research involves making qualitative and quantitative determinations of flavonoids using HPLC and chromatographic analyses, and purifying flavonoids and phenolics by chromatographic methods and characterizing them chemically using advanced level spectroscopic techniques. Particular attention will be placed on phlobaphenes and their role in postharvest after darkening of seeds. Position requires the incumbent to have a well-developed knowledge of the chemistry of secondary plant metabolites and natural products and their isolation, purification, and identification. Some knowledge in genetics is needed because of the use of genetic markers in the research. A knowledge of plants is also needed as the incumbent will be required to produce seed needed for experiments. The incumbent must have the ability to plan and conduct integrated research and to form productive collaborations with other scientists.

The postdoctoral position, a GS11/12, is funded for two years. A Ph.D. in natural products chemistry or related field with a demonstrated ability to publish is required. To apply, please send curriculum vitae, selected papers, and the names of three references to: **Dr. George L. Hosfield, USDA, ARS, Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824. Telephone: 517-355-0110; FAX: 517-337-6782.** Applications should indicate "Position Number 2W7764."

USDA/ARS is an Equal Opportunity Employer.

POSTDOCTORAL POSITIONS
Plant Molecular Biology

Two **POSTDOCTORAL POSITIONS** are currently available to work on two aspects of the soybean-*Phytophthora sojae* interaction: 1) cloning the soybean disease resistance gene *Rps1*; 2) investigating the role of the phosphoinositide signal pathway in the expression of disease resistance. Candidates with experience in soybean transformation, molecular biology, or protein biochemistry are encouraged to apply with three letters of recommendation to: **Dr. Madan K. Bhattacharyya, Staff Scientist, Plant Biology Division, Noble Foundation, P. O. Box 2180, Ardmore, OK 73402. FAX: 405-221-7380; Telephone: 405-223-5810.** *The Noble Foundation is an Equal Opportunity Employer.*

A POSTDOCTORAL POSITION is available for an individual with experience in protein/nucleic chemistry and an interest in novel regulatory mechanisms to study the unique epigenetic regulatory system of the Spm transposon (*Cell*, 77:427, 1994), which is inactivated by methylation and encodes an autoregulatory protein that reactivates its methylated promoter. Send curriculum vitae and references to: **Dr. Nina Fedoroff, Biotechnology Institute, The Pennsylvania State University, University Park, PA 16802. FAX: 814-863-1357; Email: nvf1@psu.edu.**

POSTDOCTORAL POSITIONS
Molecular Regulation of Hepatic Gene Expression

Two **POSTDOCTORAL POSITIONS** are available to study molecular mechanisms mediating effects of insulin on hepatic gene expression. One postdoctoral investigator will characterize novel DNA/protein complexes and DNA/protein interactions by methylation interference, affinity labeling, and Southwestern blotting/cloning. Another investigator will examine the role of signaling pathways in mediating insulin effects via defined response sequences, using kinase assays and transfection models. Send curriculum vitae and names and telephone numbers of three references to: **Dr. Terry Unterman, Endocrine Section (M/C 640), Department of Medicine, University of Illinois College of Medicine at Chicago, 840 South Wood Street, Chicago, IL 60612. FAX: 312-455-5877.**

POSTDOCTORAL POSITIONS

The Department Medicine/Medical Oncology of The University of Texas Health Science Center at San Antonio (UTHSCSA) has positions available for **POSTDOCTORAL** trainees in our NIH-funded breast cancer SPORE program. Areas of research include: molecular characterization of ER variants, mechanisms of drug and Tamoxifen resistance, genetic markers of premalignant disease, oncogenes and tumor suppressors in breast cancer, transgenic mouse models, signal transduction, and growth factors. Minimum requirements are: Ph.D. degree in molecular or cellular biology or M.D. with research experience, *U.S. citizen or permanent resident*. Stipend to be determined by NIH fellowship guidelines. Send curriculum vitae, letter of research interests, and names of three references to: **C. Kent Osborne, M.D., Department of Medicine/Medical Oncology, UTHSCSA, 7703 Floyd Curl Drive, San Antonio, TX 78284-7884.** *The UTHSCSA is an Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL POSITION AVAILABLE

NIH-funded (3½ years) studies on the characterization and molecular etiology of membrane glucocorticoid receptors. Will entail a broad range of techniques including cell culture, receptor modifications, binding, purification, immunocytochemistry, PCR amplification-based sequencing, and cDNA cloning. Available starting February 1, 1996; salary depends on qualifications. Send curriculum vitae and three letters of reference to: **Dr. Bahiru Gametchu, Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI 53226. Email: gametchu@post.its.mcw.edu.** *Medical College of Wisconsin is an Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL POSITIONS to apply advanced Optical Mapping approaches to human genome analysis (see *Science*, 262:110-114, 1993; *Nature Genetics*, 9:432-438, 1995; *PNAS*, 92:165-169, 1995; *C&E News*, May 15, 1995; *PNAS*, 92:5164-5168, 1995). Develop new approaches to sequencing, applications of new genotyping methodologies, whole genome approaches to restriction mapping and gene hunting. Send curriculum vitae, summary of past research, and three letters of reference to: **Dr. David C. Schwartz, New York University, W. M. Keck Laboratory for Biomolecular Imaging, Department of Chemistry, 31 Washington Place, New York, NY 10003.**

DUKE UNIVERSITY
CELL/MOLECULAR BIOLOGY

POSTDOCTORAL POSITION: Investigate the gene regulation of antioxidants and proinflammatory cytokines in models of lung injury and repair. Approaches include quantitative RT-PCR, differential display, and gene transfection. Please submit a curriculum vitae with references to: **Rodney Folz, M.D., Ph.D., Duke University Medical Center, Box 3177, Durham, NC 27710. Telephone: 919-684-2513; FAX: 919-684-3067.** *Equal Opportunity Employer.*

POSTDOCTORAL POSITION available immediately to study physiological roles for protein carboxyl methylation in animal cells. A microinjection model involving *Xenopus* oocytes is used to analyze the biochemical processing of methylated proteins and peptides. Transfected mammalian cell lines and *Drosophila* are used to study the consequences of methyltransferase manipulation on the stress response and aging. Research experience in molecular biology, mammalian cell culture, and/or protein biochemistry is preferred. Send curriculum vitae and three references to: **Dr. Clare O'Connor, Biology Department, Boston College, Chestnut Hill, MA 02167.**

POSTDOCTORAL POSITION is available beginning January 1, 1996 to study the electrophysiologic and pharmacologic consequences of gender-based differences in expression of cardiac ion channels. Applicants should have extensive experience in cardiac cellular electrophysiology, patch clamp, and cardiac myocyte cell culture techniques. Send curriculum vitae and three letters of reference to: **Raymond L. Woosley, M.D., Ph.D., Department of Pharmacology, Georgetown University Medical Center, 3900 Reservoir Road, NW, Washington, DC 20007.**

KALAMAZOO COLLEGE
Department of Biology

Kalamazoo College seeks to fill positions in molecular biology and genetics (tenure-track) and in botany and ecology (anticipated one-year sabbatical replacement). Ph.D. (or evidence of imminent completion) required. Salary competitive and consistent with level of experience. Candidates are expected to have high aptitude and interest in undergraduate teaching, a commitment to the liberal arts, and a desire to involve undergraduates in scholarship both inside and outside the classroom. Kalamazoo College is a highly selective nationally known liberal arts college with a unique and distinguished undergraduate program. The Biology Department recently moved into a new "state-of-the-art" science teaching and research facility that includes modern teaching and student/faculty research labs.

Molecular Biologist/Geneticist: tenure-track position at the Assistant Professor level preferred or commensurate with level of experience beginning September 1996. Teaching responsibilities include genetics, molecular biology, and other electives, such as microbiology or developmental biology, depending upon experience and background. Interest and ability to involve undergraduates in research are essential. Start-up funds will be made available.

Botanist/ecologist: one-year sabbatical replacement at the Assistant Professor level beginning September 1996. Teaching responsibilities include General Botany with possible participation in evolution or ecology sections of introductory courses. Other options include Ecology or Environmental Science for majors or non-majors. An excellent opportunity for an individual to develop a set of strong credentials in a program that puts a premium on excellence in undergraduate science education.

Completed applications received by November 24, 1995 will receive full consideration, with later applications reviewed as needed until the positions are filled. Send curriculum vitae (including a description of research interests), undergraduate and graduate transcripts (unofficial acceptable), a detailed statement of teaching philosophy and goals, and three letters of recommendation to: **Dr. Paul D. Olexia, Chair, Biology Department, Kalamazoo College, 1200 Academy Street, Kalamazoo, MI 49006-3295.** Please specify clearly for which position you are applying. *Kalamazoo College encourages candidates who will contribute to the cultural diversity of the College to apply and to identify themselves if they wish. Equal Opportunity Employer.*

■ Neurobiologist ■

Assistant Professor, tenure-track position available in the Department of Biology, September, 1996. Successful applicant will be expected to teach undergraduate and graduate courses in neurobiology; to participate in the development of, and teach in, a general education curriculum; to establish an externally funded research program; and to direct the research of students at the undergraduate, master's and doctoral levels. Research focus could be in areas of neurophysiology, neurochemistry, receptor biology, or molecular or developmental neurobiology. However, applications will be particularly welcome from candidates with research interests in areas such as neuroethology, neuroendocrinology, neuroplasticity and biorhythm that would enable direct participation in our doctoral track in Environmental Biology. Ph.D., postdoctoral (or equivalent professional) experience. Send letter stating interests and goals in research and teaching, CV and three reference letters to: **Dr. Richard H. White, Chair, Biology Search Committee, Biology Department, University of Massachusetts Boston, 100 Morrissey Blvd., Boston, MA 02125-3393. Application deadline is November 20, 1995.** An Affirmative Action, Equal Opportunity, Title IX employer.

University of Massachusetts
Boston

Scripps Institution of Oceanography
Center for Clouds, Chemistry, and Climate

Scripps Institution of Oceanography (SIO) at the University of California, San Diego (UCSD) invites applications for appointment to a research position in the area of atmospheric chemistry.

The research appointments parallel University of California faculty appointments, but are without teaching requirements. For this atmospheric chemistry position applications for the appointment at assistant, associate, or full research scientist will be considered. Salary is commensurate with experience and based on the University of California salary scale. Partial salary support will be provided from various sources subject to availability of funds.

Candidates must hold a Ph.D and have demonstrated their ability to conduct high quality research. Research area may include chemistry of tropospheric games and aerosols, with an emphasis on atmospheric observations. The successful candidate will be expected to establish an extramurally funded research program and to involve SIO graduate students. There will be opportunities to interact with an international and interdisciplinary group of researchers in clouds and climate,. and to participate in on-going field experiments involving aircraft, ships, and surface observations.

Closing date is January 31, 1996. Applicants should send a curriculum vitae, selected reprints, a list of at least three references and a brief statement of research interests to:

V. Ramanathan, Director
Center for Clouds, Chemistry, and Climate
Scripps Institution of Oceanography
University of California, San Diego
9500 Gilman Drive
Mail Code 0239
La Jolla, CA 92093-0239

The University of California is an equal opportunity employer.

ASSISTANT PROFESSOR
FRESHWATER/LANDSCAPE ECOLOGY
Beginning Academic Year 1996-1997

The Harvard University Graduate School of Design has an Assistant Professor position available beginning in the academic year 1996-1997 for a dynamic individual qualified to offer graduate-level instruction in freshwater/landscape ecology. This full-time position may be filled for a fixed initial term, normally of three years, with responsibilities for teaching and scholarship. Candidates should have a record of scholarly research productivity on land/water interactions, an ability to relate research to the design professions, and some teaching experience. A Ph.D and published research in ecology with a focus on streams, rivers, wetlands, or lakes are required. Experience in landscape ecology, aquatic restoration, hydrology, or working with landscape architects and planners is desirable.

Applications are invited before 15 December, 1995 on the application forms available from: Office of Faculty Planning, Harvard University Graduate School of Design, 48 Quincy Street, S203, Cambridge MA 02138, Attn: Richard T. Forman. Fax: (617) 496-5310. Applicants should also include copies of their most relevant publications with their applications.

Harvard is an Equal Opportunity/Affirmative Action Employer.

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HARVARD UNIVERSITY

Graduate School of Design

POSITIONS OPEN

POSTDOCTORAL POSITION available immediately for an NIH-funded study of genes and proteins involved in alginate biosynthesis/degradation in *Pseudomonas aeruginosa*. Applicants must possess expertise in gene cloning, sequencing and expression, and purification of recombinant proteins. Candidates should send curriculum vitae, reprints, and names of three references to: **Neal L. Schiller, Ph.D., Chair, Graduate Program in Microbiology, University of California, Riverside, CA 92521-0121. FAX: 909-787-5504; Email: neals@ucrac1.ucr.edu.** *Affirmative Action/Equal Opportunity Employer.*

MOLECULAR TOXICOLOGY
UNIVERSITY OF WISCONSIN-MADISON

POSTDOCTORAL POSITIONS in Molecular, Cellular and Biochemical Toxicology funded by an NIEHS training grant.

Topic areas include: regulation of gene expression (toxicant metabolizing enzymes, oncogenes, DNA repair enzymes); cellular effects mediated by the Ah-receptor; toxicant activation; toxicity in developing organisms; mechanisms of carcinogenesis; action of toxicants on liver, kidney, gonadal, nerve, and immune cells.

Time will be allocated for training in Toxicology, and Molecular and Cellular Biology as appropriate. Collaborative projects involving more than one laboratory are encouraged.

Applicants must be U.S. citizens or permanent residents and should send a curriculum vitae, three references (addresses and telephone numbers), and a letter stating research interests to: **Dr. Colin R. Jefcoate, Director, Environmental Toxicology Center, B157 Steenbock Library, 550 Babcock Drive, Madison, WI 53706. Telephone: 608-263-4580.** *An Equal Opportunity Employer.*

BIOLOGY ONE-YEAR SABBATICAL REPLACEMENT

Earlham College seeks a one-year replacement to teach Human Anatomy and Physiology, Genetics, and related introductory courses. Ph.D. or A.B.D. Should send transcripts, curriculum vitae, three letters of reference that speak to teaching effectiveness, and a letter of application describing biology teaching experience and philosophy of teaching to: **Jerry Woolpy, Earlham College, Richmond, IN 47374.** Apply before January 15, 1996. *Earlham College is an Affirmative Action/Equal Opportunity Employer. We particularly encourage applications from women, minorities, and Quakers.*

SCIENTIFIC LIBRARIAN

Progressive Marina del Rey nutrition firm seeks **CLINICAL RESEARCH ASSOCIATE** or **LIBRARY RESEARCHER** for cutting edge work on health and nutrition. If you're fascinated by the new trend in nutritional support, alternative health care, and life extension, this firm is the answer. Excellent opportunity for alternative-care minded individuals with an eye on the future. Send résumé to: **Ning Li, 520 Washington Boulevard, Suite 420, Marina del Rey, CA 90292.**

RESEARCH ASSOCIATE needed by southwest Ohio University to conduct multidisciplinary ergonomics-biomechanics research studying the risks of slips and falls on elevated and/or inclined surfaces, the ergonomics of task performances on slippery surfaces, and the gait and balance in lead exposed children. Coordinates data collection with human subjects developing protocols and questionnaires. Writes computer programs in Asyst and C++ to collect, analyze, graph, and plot data using software and hardware from AMTI Force Platforms, Peak Performance System and FScan Pressure Sensors. Uses SAS programming and multivariate regression analysis to analyze results and writes reports for scholarly journals. Must have Ph.D., or completed all degree requirements, in environmental health or industrial hygiene. Must have two years of experience in job described or two years of experience or dissertation involving writing programs in C++ and Asyst to collect and analyze data using AMTI Force Platforms, Peak Performance System and FScan Pressure Sensors and working with human subjects. Experience may be gained before, during or after degree and may be gained concurrently. Work schedule: forty hours per week, 8am to 5pm, Monday through Friday. Salary: $39,140.04 per year. *Must have proof of legal authority to work permanently in the U.S.* Send two résumés and cover letters (no calls) to: **T. Do, Reference No. 1357, Ohio Bureau of Employment Services, P.O. Box 1618, Columbus, OH 43216.**

POSITIONS OPEN

FOOTHILL DE ANZA COMMUNITY DISTRICT
Los Altos Hills, California

BIOTECHNOLOGY INSTRUCTOR

This position will be responsible for teaching biotechnology, microbiology, and cell biology along with the appropriate equipment and techniques used in microbiology and biotech labs. Master's degree required. Application and job description may be obtained from:

Employment Services
Telephone: 415-949-6217 or
Email: cms6438@mercury.fhda.edu

A résumé or curriculum vitae may not be substituted for a completed application. *Affirmative Action/Equal Opportunity Employer.*

RESEARCH ASSISTANT I

Coronary vascular physiology and pharmacology research. Primarily responsible for conducting both functional and imaging studies of coronary arteries obtained from various species of animal as well as human hearts. Responsible also for implementation of highly integrated and computerized data acquisition system to correlate magnetic resonance imaging (MRI) of coronary arteries to their functional reactivity. Will assist in the experimental designs, data analyses, and preparation of reports and publishable manuscripts. Requires B.S. in biology and/or computer engineering plus one year of experience in duties described or one year of experience in computer engineering or medical engineering. Computer engineering or medical engineering experience must include controlling of computer based data acquisition/processing techniques, analog/digital equipment experience, and ability to program for data processing, and knowledge of MRI technique. Salary: $22,000 per calendar year. Work 8am to 5pm, 40 hours per week. *Applicants must be authorized to work in the United States.* Résumés to: **M. Fowler, Alabama State Employment Service, P. O. Box 12046, Birmingham, AL 35202-2046. Refer to Job Order Number AL 5020747.** *Equal Employment Opportunity.*

PLANT TRANSFORMATION

DNA Plant Technology Corporation has an immediate opening in its Vegetable Tissue Culture Group for an experienced **SCIENTIST** to work on dicot transformation. Current research is in the development of transformation systems in a variety of vegetable crops and the production of new transgenic lines for commercial release. Candidates must have transformation experience using *Agrobacterium*, preferably in the development or trouble shooting of transformation protocols. Experience with the tissue culture and transformation of tomato and/or strawberry would be valuable.

DNAP offers an excellent compensation and benefits package that includes stock incentives. Candidates should send a résumé with three references to: **Dr. Alison Morgan, DNA Plant Technology Corporation, 6701 San Pablo Avenue, Oakland, CA 94608. FAX: 510-547-2817; Email: morgan@dnap.com.** *An Equal Opportunity Employer.*

SENIOR RESEARCH ASSOCIATE

The Department of Neurology, Columbia University College of Physicians and Surgeons, seeks a **SENIOR RESEARCH ASSOCIATE** to assist in laboratory work in neurogenetics. Responsibilities will include DNA sequencing and sequence analysis, mutation analysis, RNA and protein extraction and assay. M.D. or Ph.D. required, with at least three years of postdoctoral experience in the necessary techniques. Send curriculum vitae to: **Torbjoern G. Nygaard, M.D., Department of Neurology, Columbia University, 710 West 168th Street, New York, NY 10032.**

Columbia University is an Affirmative Action/Equal Opportunity Employer.

POSITIONS OPEN

THE CLEVELAND CLINIC FOUNDATION

STAFF SCIENTISTS
PROGRAM IN MACULAR DEGENERATION AND INHERITED RETINAL DISEASES
Division of Ophthalmology
The Cleveland Clinic Foundation (CCF)

Recruitment of **STAFF SCIENTISTS** is underway for the newly expanding Eye Institute. Two positions are available immediately at the Assistant, Associate or Full Scientist level, and three additional positions will be filled in the fall of 1997. Basic investigators with training in molecular biology, cell biology, biochemistry, human genetics, or epidemiology, with research interests that impact the clinical areas of macular degeneration and inherited retinal diseases, will be seriously considered. Applicants should send curriculum vitae; names of three references; and a statement of past research activities, current interests, and career goals to: **Joe G. Hollyfield, Ph.D., Director of Ophthalmic Research, Research Institute FFb, The Cleveland Clinic Foundation, Cleveland, OH 44195-5245. FAX: 216-445-3670. Email: <hollyfj@ccsmtp.ccf.org>.** *CCF is an Equal Opportunity/Affirmative Action Employer.*

OPEN EXAMINATION FOR SENIOR TOXICOLOGIST

The California Environmental Protection Agency, Department of Pesticide Regulation, seeks a **SENIOR TOXICOLOGIST** in the pesticide regulatory program. Requirements: four years of postdoctoral experience in toxicology or closely related field. This experience must include the interpretation of toxicological findings relative to probable human health and one year of experience in the development and design of toxicological research and investigative studies. The senior toxicologist position is responsible for both supervisory tasks and may perform the most difficult or sensitive work. The position is located in Sacramento. Salary range: $5,242 to $6,336 per month. Comprehensive benefits package. Submit application and résumé to the address below by the Final File Date: November 30, 1995.

Department of Pesticide Regulation
Personnel/Examination Unit
1020 N Street, Room 126
Sacramento, CA 95814
Attn: SDJ

For State application and announcement **Telephone: 916-322-4553.**

RELOCATION OF RESEARCH PROGRAMS TAMPA BAY RESEARCH INSTITUTE

The Tampa Bay Research Institute (TBRI) invites applications from established **SCIENTISTS** to relocate funded research programs in Molecular Biology, Cancer Research, Virology, and Gene Therapy. TBRI is an independent, nonprofit research organization located in the beautiful Tampa Bay Area. The institute is based in a modern facility with excellent individual laboratories suitable for molecular biology/biotechnology research in a variety of areas of modern biology. Individuals with funded research programs in the fields of interest are encouraged to apply. Please send application, including a statement of research interest and names and addresses of three referees, to: **The Chair, Search Committee, Tampa Bay Research Institute, 10900 Roosevelt Boulevard, St. Petersburg, FL 33716.**

COURSES AND TRAINING

ATTENTION PH.D.'S AND M.S.'S

Earn M.D. degree from top ranked international medical school. Alternate studies program in English, two years basic science, three weeks on six weeks off. World Health Organization listed and Educational Committee on Foreign Medical Graduates approved. Eligible for third and fourth years clinical studies in USA. Contact: **Dr. Alan Butkowsky. Telephone: 203-563-4554.** Total cost per year is approximately $18,000.

Animal Health Research

Pfizer, a leader in animal health products has established a reputation for success in finding/developing breakthrough products for the global market. Our new Molecular Biology laboratory is a dynamic part of Pfizer's Animal Health discovery operation in Lincoln, Nebraska.

At Pfizer we draw from real life.

Currently, Pfizer's cutting-edge Molecular Biology department, has an outstanding opportunity for a team-oriented, technically talented:

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Design and conduct contemporary experiments on RNA viruses for the discovery of biological animal vaccines. To qualify, you must have a Ph.D. or equivalent in microbiology or biochemisty with at least 2 years of relevant postdoctoral experience with RNA viruses, including retroviruses or coronaviruses, with the ability to manipulate RNA using contemporary techniques.

In addition to the opportunities to interact with one of the world's finest corporate scientific communities, you will enjoy the many recreational and educational amenities of our community and its friendly, family-oriented Midwestern lifestyle. Relocation assistance is available. Please send detailed resume to: **Employee Resources, Biological R&D, 601 West Cornhusker Highway, Lincoln, NE 68521.**

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Cardiovascular Pharmacology

Investigator

SmithKline Beecham, a worldwide leader in pharmaceutical research, has an excellent research opportunity in our Cardiovascular Pharmacology department for an Investigator with good understanding of molecular biology techniques and strategies and special emphasis on transcription regulation/factors.

While working in a very interactive and collaborative environment, the successful candidate will establish independent basic research in areas such as atherosclerosis, tissue remodeling and matrix formation, smooth muscle/endothelial cell biology and vascular functions. Responsibilities will also include supervising an associate scientist and taking care of administrative aspects of lab work.

Qualified individuals will have a PhD in Biological Sciences, at least one full term of post-doctorate fellowship, and 5-7 years' experience in independent scientific research in an academic or pharmaceutical research organization. Strong experience in gene cloning, yeast dihybrid systems and cell biology are important. A strong track record of publication in high-quality, peer-reviewed professional journals is mandatory.

Our state-of-the-art research facility is located in suburban Philadelphia. We offer a competitive compensation/benefits/relocation package and a stimulating team environment. For confidential consideration, send your resume to: SmithKline Beecham Pharmaceuticals, Job Code H5-0107, P.O. Box 2645, Bala Cynwyd, PA 19004 . We are an Equal Opportunity Employer, M/F/D/V.

SB

SmithKline Beecham

Pharmaceuticals

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TWO FACULTY POSITIONS
DEPARTMENT OF BIOCHEMISTRY AND BIOPHYSICS
IOWA STATE UNIVERSITY

The department seeks to fill two tenure-track appointments at the level of assistant professor. The successful applicants will be expected to develop independent research programs that emphasize biochemical and/or biophysical aspects of important biological problems. Applicants should also have an interest in graduate and undergraduate teaching. Generous start-up funds and space in the new Molecular Biology Building will be available.

Applicants should send a curriculum vitae and description of research accomplishments and interests, and have three letters of recommendation sent to:

Faculty Search Committee
Dr. Robert Thornburg, Chairman
Department of Biochemistry and Biophysics
1210 Molecular Biology Building
Iowa State University
Ames, IA 50011

To ensure full consideration, all materials should be received by January 15, 1996. *Iowa State University is an affirmative Action/Equal Opportunity Employer. Applications from women and minority candidates are especially encouraged.* For additional information about the Department and Iowa State University see: http://molebio.iastate.edu/bbhtml/homepage.htm

DIRECTOR

Research Animal Resource Center

Memorial Sloan-Kettering Cancer Center and the Cornell University Medical College

The Memorial Sloan-Kettering Cancer Center (MSK) and the Cornell University Medical College (CUMC) are seeking a Director for their jointly-operated Research Animal Resource Center (RARC) which encompasses approximately 35,000 NSF with an average daily census of more than 32,000 animals. The Director of RARC has direct responsibility for managing an AAALAC-accredited program of laboratory animal care and use that complies with all federal, state and local animal welfare regulations. The Director serves as the chief veterinarian for both institutions and has MSK/CUMC-wide responsibility for the development, recommendation and implementation of policies regarding the care and management of research animals.

Responsibilities include supervising two veterinarians and approximately 29 managerial and associated support staff members in the General Animal Facilities and Animal Health Divisions who provide clinical care, post-operative care, anatomic and clinical pathology services, and related professional services. The annual budget of RARC exceeds $2.5M.

Candidates must have either the DVM or VMD from a recognized school of veterinary medicine; must be Board certified by the American College of Laboratory Animal Medicine and must have approximately 10 years of significant administrative experience (including personnel and budget management) in the care and managing of research animals; should have research experience.

Individuals should submit a letter of interest, a curriculum vitae, and the names of three references to: **Senior Vice President, Memorial Sloan-Kettering Cancer Center, Room 1308H, 1275 York Avenue, New York, NY 10021.**

Memorial Sloan-Kettering Cancer Center and the Cornell University Medical College are committed to the policy that all persons shall have equal access to its programs, facilities and employment without regard to race, color, creed, religion, national origin, sex, age, marital status, disability, public assistance status, veteran status, or sexual orientation.

NEDO

Public Announcement Regarding the Recruitment of Researchers for the NEDO Industrial Technology Fellowship Program in FY1996.

The New Energy and Industrial Technology Development Organization (NEDO) has been conducting research and development on new energy, energy conservation and industrial technologies. In order to ensure efficient promotion of its research and development projects, NEDO would like to recruit researchers to participate in such projects.

Those who wish to participate in NEDO's projects should apply in accordance with the following information.

1. Purposes and Activities

In order to more efficiently conduct NEDO's ongoing research and development projects, NEDO will employ researchers (hereinafter "Industrial Technology Researchers") to be seconded to organizations which NEDO has entrusted with its projects to be engaged in project research and development work.

2. Projects

Please refer to the attachment entitled "Projects for Which Industrial Technology Researchers Will Be Enlisted."

3. Employment Period

The employment period will be from April 1, 1996 to March 31, 1997. (The total employment period may extend up to a maximum of three consecutive years, that is, up to March 31, 1999, based on an evaluation at the end of each fiscal year.)

4. Number of Researchers to be Recruited.

Several researchers will be recruited.

5. Requirements

(1) A person who holds a doctorate degree or who has a comparable level of research ability;

(2) A person without any health problems which may constitute an obstacle to research activities;

(3) A person whose Japanese or English language ability does not create any obstacle to his/her life in Japan.

6. Work Conditions, Etc.

(1) The status of individual Industrial Technology Researchers shall be as a full-time employee on a contract basis.

(2) Work conditions shall be in accordance with NEDO's work regulations.

(3) NEDO will pay a total annual remuneration of approximately ¥7.0 million based on regulations provided for by NEDO. (This amount includes basic salary, benefits, taxes and the researcher's share of premiums for social insurance. Payments equal to 1/12 of the total amount of annual remuneration will be made on a monthly basis.)

(4) NEDO will pay the actual amount of transportation expenses necessary for individual Industrial Technology Researchers to travel from their home or place of residence to their place of work in Japan. (The amount shall not include expenses for anyone other than individual Industrial Technology Researchers.)

7. Screening of Successful Applicants

Successful applicants will be selected by NEDO's internal committee comprised of such people as those with knowledge and experience in the field of industrial technology.

8. How to Apply

Those who wish to apply are requested to send NEDO an Application for an Industrial Technology Researcher Position in FY1996 with necessary information filled in conforming to Format 1 attached hereto together with the documents listed below.

(1) Curriculum vitae (There is no particular requirement for the format. The document should be typed and signed by the applicant.)

(2) Copy of diploma (only of the last educational institution)

(3) Copy of certificate of doctorate degree

(4) List of research publications (papers and oral presentations) (See Attachment 2)

(5) Copies of two or three representative research papers (Excluding doctorate thesis)

9. Deadline for Submitting Applications

All applications should be forwarded to the person indicated below and arrive at NEDO no later than December 1, 1995.

Shunji Kubota
Research and Development Division
Industrial Technology Department
New Energy and Industrial Technology Development Organization
3-1-1, Higashi-ikebukuro, Toshima-ku, Tokyo, 107 Japan
Telefax: 03-3981-1536

10. Inquiries

For inquiries regarding this program, please contact either of the following persons at NEDO by telefax:

Shunji Kubota
Research and Development Division
Industrial Technology Department
Telefax: 03-3981-1536

Masamichi Yamamoto
Policy Planning and Coordination Division
Planning Division
Telefax: 03-3981-1059

*The information given above is based on the assumption that approval will be obtained from the national budget. Please note, therefore, that the information is subject to change depending on the situation related to the budget approval.

NEDO

(Format 1)

Application for an Industrial Technology Researcher Position in FY1996

Date:

To: Mr. Hachio Iwasaki
New Energy and Industrial Technology
Development Organization

Name and address of applicant:
Signature

Regarding the subject shown above, I hereby wish to apply for a position as follows:

Name of applicant:
Date of birth: (Age:) Male/female
Nationality:
Home address: Tel. or fax no. Office address: Tel. or fax no.
Name of project the applicant wishes to participate in:
Academic background: Month, year Month, year Month, year
Professional career: From (Month, year) to (Month, year) From (Month, year) to (Month, year) From (Month, year) to (Month, year)
Qualifications:
Research career: From (Month, year) to (Month, year) research on From (Month, year) to (Month, year) research on From (Month, year) to (Month, year) research on
Awards: Month, year Received XXX Prize (for research on) Month, year

(Format 2) List of Research Presentations/Publications

(Name of applicant)

Date	Classification	Title	Medium	Name(s) of co-authors	Remarks

(Note) 1. This document shall be prepared using JIS A4 or letter size paper.
2. In the column entitled "Classification," either "Written" or "Oral" should be entered.
3. The names of co-authors of papers should be written in the same order as given in the papers concerned.
4. In the column entitled "Remarks", enter 1, 2 or 3 for a publication made in a magazine for an academic society, a business magazine or other media, respectively.

Reference Person Name: Affiliation: Tel. or fax no.:

(Note) 1. This document shall be prepared using Japan Industrial Standard (JIS) A4 paper (length: 29.7 cm × width: 21 cm) or letter size paper.
2. The application may be prepared on more than one page to cover all necessary items.
3. The reference person shall be a person with knowledge about such issues as the contents of the applicant's research or achievements.

"Projects for Which Industrial Technology Researchers Will Be Enlisted."

1. *Marine Biotechnology* (Fine Chemicals from Marine Organisms and Tropical *Bioresources*)

The goal of this R&D project is to establish technology for the production of various fine chemicals by promoting the use of unexplored marine and tropical organisms and by developing new basic relating biotechnologies.

2. Ultimate Manipulation of Atoms and Molecules

In combination with the mechanical probe technique and beam technique, the new technology allows the identification, observation, measurement and manipulation of atoms and molecules on surface of various matter, organic molecules such as DNA, and atom assembles in free space. R&D of simulation technology will also be pursued to exactly predict atomic and molecular processes and their properties on purely theoretical basis.

3. Synergy Ceramics

Material properties and functions are closely related to the type and structure of the constituent elements. The microstructure of ceramic materials is composed of many kinds of structural elements such as chemical bonding, crystalline grains, fibers, and layers. These elements have different sizes and morphologies (shape, configuration, distribution, etc.), and their size can range from the atomic-molecular scale through the nano-scale (1/1,000,000 mm), micro-scale (1/1,000 mm) to the macro-scale.

4. International Clean Energy Network Using Hydrogen Conversion

International Clean Energy Network Using Hydrogen Conversion System (World Energy Network, WE-NET) is a large scale project which will provide a comprehensive solution to the global dilemma of producing and utilizing energy while simultaneously preserving the environment. The WE-NET project will construct a clean energy network on a worldwide scale, where hydrogen will be produced by employing various kinds of renewable energies such as hydropower, solar, geothermal and wind power in areas blessed with those energies. The produced hydrogen will then be transported to and used in high energy consumption regions.

5. Broad Area Energy Utilization Network System (Eco-Energy City)

The Eco-Energy City Project is a multi-technology initiative which aims at promoting the development of innovative technologies necessary for constructing broad-area energy networks. Echo-Energy Cities will have low energy demand (energy conservation) and little negative effect on the environment. In order to promote the Eco-Energy City Project a wide range of innovative elemental technologies such as waste heat recovery and energy conversion technologies, energy transportation technologies, energy storage technologies, heat utilization technologies, environmental protection technologies, system technologies and etc, have being developed from FY1993.

CRCLEME

Cooperative Research Centre for Landscape Evolution & Mineral Exploration

The Cooperative Research Centre for Landscape Evolution and Mineral Exploration (CRC LEME) is an unincorporated joint venture between Australian National University, University of Canberra, Australian Geological Survey Organisation and CSIRO Exploration and Mining

Established on 1 July 1995, CRC LEME's vision is the discovery of concealed world-class ore deposits through knowledge of Australian landscape evolution.

The broad mission of CRC LEME is to develop an enhanced understanding of the three-dimensional Australian landscape in terms of its geomorphology, regolith geology, weathering diagenesis and geochemistry, and translate this understanding into vastly improved methods for finding world-class mineral deposits, and into the training of geoscientists.

WITHIN THIS BROAD MISSION THERE ARE A NUMBER OF OBJECTIVES:

- To establish the spatial relationships between regolith, landforms, and bedrock
- To establish uniform terminology
- To understand the relationships between weathering processes and evolutionary stages of regolith development
- To develop appropriate exploration procedures for different landscape situations
- To ensure the effective transfer of knowledge to the exploration industry, academia and the wider community of interested professionals

CRC LEME HAS THE FOLLOWING PROGRAMS:

1 Regolith and landscapes of the Australian Shield
2 Regolith and landscapes of the Tasman Fold Belt
3 Regolith and landscapes of the Australian sedimentary basins
4 Synthesis of Australian regolith-landscape evolution

Education and Applications are important activities of CRC LEME.

To undertake the work of CRC LEME the following position is now available at the Australian Geological Survey Organisation.

Program Leader

(Principal Research Scientist)
A$66,472 – A$74,884

AGSO
AUSTRALIAN GEOLOGICAL SURVEY ORGANISATION

CRC LEME is seeking to appoint a highly motivated Geoscientist to work in Program 3, Regolith and Landscapes of Australian Sedimentary Basins. The person we appoint will have a PhD or equivalent in geoscience with expertise in the broad area of regolith studies and familiarity with at least some of the following:

- geomorphology
- supergene ore deposits
- regolith geochemistry
- terrestrial sedimentology
- surficial geology

Experience in mapping regolith, landforms or soils would also be an advantage. The appointee will have a commitment to mineral exploration. We will also be looking for experience in project management.

The position will be based at AGSO in Canberra with regular travel to Sydney and occasional travel throughout Australia. The job will involve leadership of Program 3, the development and leadership of research projects within the Program, and liaison with industry representatives. The appointee will lead a team of scientists and professionals from government, universities and industry. He/she will be expected to make a contribution to the development of research directions in the CRC LEME.

The appointment will be permanent, subject to normal probationary conditions. Australian Government superannuation is available.

Further information regarding this position is available from Dr Colin Pain, AGSO, telephone 61 6 249 9469, or Dr Raymond Smith, Director, CRC LEME, telephone 61 9 387 0272.

Copies of the Duty Statement and Selection Criteria are available from the receptionist on telephone 61 6 249 9808.

Please send your application to the Personnel Manager, AGSO, Box 378 Canberra ACT 2601 AUSTRALIA. Applications should be marked "Position: PRS/1999" and be received by close of business on Friday 1 December 1995.

AGSO is an equal opportunity employer.

UNIVERSITY OF TASMANIA

BURNIE

Chair in Dairy Science Department of Agricultural Science

Applications are invited for appointment to the position of Professor of Dairy Science within the Department of Agricultural Science at the University of Tasmania. This position will be based at the University's North-West Campus in Burnie and the initial appointment will be for a period of 5 years.

The successful applicant will have a distinguished international research reputation in animal nutrition aspects of dairy research. Applicants with research interests in the area of pasture-based ruminant nutrition are particularly encouraged to apply for this position.

As a staff member of the Department of Agricultural Science, the appointee will function as the Director of the University's Cuthbertson Research Laboratories and will be responsible for developing and leading the University's research, education and postgraduate programs in dairy science. The appointee will join dairy researchers and technical support staff of the Tasmanian Institute of Agricultural Research (TIAR), a multi disciplinary team of researchers located within the North-West Centre. This research group will be responsible for delivering industry prioritised research to support the rapidly expanding dairy industry in Tasmania, in accordance with research strategies endorsed by the Board of the TIAR. Resources to support this research will be provided by the University, the Department of Primary Industry and Fisheries (DPIF), the Dairy Research and Development Corporation and industry.

Facilities at Burnie include the recently completed Cuthbertson Research Laboratories, the DPIF Elliott Research Station and the TAFE/University Farm.

The appointee will be expected to contribute to all academic activities of the University and the further development of collaborations between the University, DPIF, TAFE and industry in all areas of dairy science education, research and training.

Further details concerning the post, the appointment criteria and general information about the University are available from the Secretary, Personnel Services, telephone 61-03-24 3537, facsimile 61-03-24 3437. Details on the academic programs within the Department of Agricultural Science and the current and planned collaboration with the TIAR, TAFE and industry, are available from Professor RJ Clark, Head of Department, Agricultural Science, University of Tasmania, Hobart, Tasmania 7001, Australia, telephone 61-02-20 2620, fax 61-02-20 2642, email Rob.Clark@agsci.utas.edu.au.

The present professorial salary is $AU80,176 pa plus superannuation. With the consent of the University, academic staff may also undertake consultative work.

Applications, quoting reference number BA 125/95 and including the names of three referees, should give particular attention to the selection criteria and reach the Director of Personnel Services, University of Tasmania, PO Box 1214, Launceston, Tasmania 7250, Australia by 15 November 1995.

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LONG-TERM POSITIONS
IN-DEPTH RESEARCH
INTERNATIONAL EXPERIENCE

The Research Development Corporation of Japan, a statutory agency of the Japanese Government, invites applications for its new ERATO projects listed below from young scientists interested in recognized, well-funded, multidisciplinary research. ERATO projects operate for five-year terms and are independent from the Project Directors' home institutions.

Masumoto SINGLE QUANTUM DOT Project

Fields: SEMICONDUCTOR OPTICAL PHYSICS, LASER SPECTROSCOPY, OPTOELECTRONICS, SURFACE SCIENCE, MATERIALS SCIENCE, CHEMISTRY
Research: Optical properties of semiconductor quantum dots, laser spectroscopy of a single quantum dot, ultrafast carrier dynamics, fabrication, theoretical studies
Director: Dr. Yasuaki Masumoto, University of Tsukuba

Kato CYTOPROTEIN NETWORK Project

Fields: MOLECULAR GENETICS, BIOCHEMISTRY, PROTEIN CHEMISTRY AND PHYSICS, CELL BIOLOGY
Research: Systems approach to the proteins of the intracellular network using full-length cDNA libraries and related tools
Director: Dr. Seishi Kato, Sagami Chemical Research Center

Doi BIOASYMMETRY Project

Fields: THEORETICAL BIOLOGY AND GENETICS, MOLECULAR GENETICS, EXPERIMENTAL EVOLUTION, DEVELOPMENTAL BIOLOGY
Research: Asymmetric replication of DNA and its role in cell differentiation and tissue development
Director: Dr. Hirofumi Doi, Fujitsu Laboratories

Mikoshiba CALCIOSIGNAL NET Project

Fields: BIOCHEMISTRY, CELL BIOLOGY, ELECTROPHYSIOLOGY
Research: The calcium-inositol trisphosphate signal pathways and their relation to functional molecules and membrane dynamics, calcium ion dynamics and information control, membrane-cytoskeletal associtations, protein targeting
Director: Professor Katsuhiko Mikoshiba, The University of Tokyo and the Institute for Physical and Chemical Research (RIKEN)

Information Qualifications Applications

Deadline: 1 February 1996

The **1995 ERATO GUIDE FOR PROSPECTIVE RESEARCHERS** is available on the Internet (**http://www.jrdc.go.jp/ERATO/NewPositions/**) or from the ERATO Overseas Representative. Applicants must hold a **Ph.D. or equivalent** degree with **less than 5 years of postdoctoral experience**, be willing to **stay at least one year**, and be **fluent in English or Japanese**. Applications must include **1) a letter describing background and interests (important!), 2) curriculum vitae, 3) list of publications, 4) copies of representative publications, and 5) names and addresses of references.** Send applications by post only, not by electronic mail.

Send Applications To

ERATO Overseas Representative, 950 Conestoga Road, Rosemont, Pennsylvania 19010-1347 USA
Voice: +1-610-527-4538, Fax: +1-610-527-2041, Internet: engel@jrdc.go.jp

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AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

THE CHINESE UNIVERSITY OF HONG KONG

Department of Biochemistry invites applications for:-
Lecturer (Human Genetics/Oncology) (carrying the title of **Assistant Professor** or **Associate Professor**, as appropriate) **(Ref. 95/115(665)/2)**

Candidates should have a PhD degree and post-doctoral experience in (i) human genetics, with substantial research emphasis on the genes responsible for inherited and somatic diseases or (ii) cancer research, particularly as it relates to the interaction among drugs, radiation and growth factors in suppressing tumour growth. The successful applicant is expected to develop externally funded research programmes and participate in graduate training and the instruction of undergraduate science and medical students. The appointee will be provided with laboratory space and start-up funds in a well appointed Department which offers tissue/cell culture facilities and analytical equipment including automated DNA sequencer, DNA synthesizer, flow cytometer, confocal microscope, mass spectrometer as well as opportunities for collaborative work. The specific area of research is open but demonstrable expertise in genome mapping and gene knock-out which can complement the Department's existing research interests will be decided advantages. English and/or Chinese are used in teaching and administration at the University.

Annual Salary and Fringe Benefits
HK$454,200 - 758,700 by 10 increments
(approx. exchange rate in September 1995: £1 = HK$12.2)
Starting salary will be commensurate with qualifications and experience.

Benefits include leave with full-pay, medical and dental care, education allowance for children, housing benefit for eligible appointee (with appointee contributing 7.5% of salary towards the provision of housing). Appointment may be made either on regular or fixed term contract. Under regular contract, retirement benefits are provided by the University. Fixed term contract also carries where appropriate a contract-end gratuity (15% of basic salary).

Application Procedure :
Send full resume in duplicate, and names and addresses of three referees, with copies of academic credentials (in duplicate) and recent publications, to the Personnel Office, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong (Fax: (852)2603 6852) before **December 8, 1995.** Please quote the reference no. and mark "Recruitment" on cover.

The United Arab Emirates University
Faculty of Science
Department of Biology
FACULTY POSITIONS

The Department of Biology at the UAE University invites applications for a faculty position in the following areas:

1. Plant Physiology (Desert Ecophysiology)
2. Developmental Biology
3. Recombinant DNA Biotechnology
4. Human/Animal Physiology
   (Special consideration is given to Cardiovascular Physiology
5. Comparative Anatomy
6. Embryology (Animal Tissue Culture)

Candidates must have a Ph.D. and demonstrate interest and potential for excellence in both teaching and research. Excellent command of Arabic and English languages is required. Applications should be received prior to December 15, 1995. Send resume with three references and letter of application to: **Secretary General, The UAE University, P.O. Box: 15551, Al Ain, Arab Emirates, Fax #(971-3)-645277.**

The Institut fur Pflanzengenetik und Kulturpflanzenforschung (IPK) Gatersleben and the Martin-Luther-Universität Halle-Wittenberg invites applications for the position of

Head of the IPK Gatersleben Genebank

(Bes.Gr. C4)

Responsibilities include teaching in the field of Biology and Genetics of Plant Genetic resources at the University in Halle.

The IPK in Gatersleben is a research centre with over 400 employees. It is funded as a Blue List institute by the federal state of Sachsen-Anhalt and the Federal Republic of Germany.

The responsibilities of the head of the genebank will include further development of the collections, their characterization and evaluation within the framework of expanding cooperations, the improvement of information systems and the increasing use of the collections in co-operative research with other departments of the IPK deploying up-to-date methods and technologies. The successful candidate will support the Federal Government of Germany in the field of plant genetic resources in national and international committees.

We are looking for an internationally recognized scientist with experience in leading interdisciplinary research groups.

The appointee will become a member of the board of directors of the IPK.

The IPK promotes equal opportunities and women are particularly encouraged to apply.

Please send a curriculum vitae, a list of publications with up to 10 reprints and names and addresses of three referees within six weeks to:

Dekan des Fachberelches Biologie
der Martin-Luther-Universität Halle-Wittenberg
Domplatz 4, D-06108 Halle/Germany

UNIVERSITY COLLEGE LONDON
Department of Human Communication Science

Chair of Communication Disorders

UCL intends to make an appointment to a newly established Chair of Communication Disorders. The Department of Human Communication Science is a new multidisciplinary department within the Faculty of Life Sciences, created by the recent transfer to UCL of the National Hospital's College of Speech Sciences. It is the largest and among the most research-orientated of its kind in the UK. The Department already enjoys close teaching and research links with other departments within UCL, and also with the Institute of Neurology/National Hospital for Neurology and Neurosurgery. There are excellent opportunities for clinical research.

We are seeking an established scholar with an international profile, who will strengthen the research ethos of the new Department, and who has a commitment to research into any aspect of communication disorders in children or adults. The main focus of the post will be on research and postgraduate teaching, with minimal administrative duties.

Further particulars may be obtained from the Head of Department, Dr Bill Wells, Chandler House, 2 Wakefield Street, London WC1N 1PG, UK. Tel 0171 837 0113. Fax 0171 713 0861. E-mail bill.wells@ucl.ac.uk. Applications (ten copies from UK candidates, one copy from overseas candidates) including a curriculum vitae and the names and addresses of three referees (including at least one overseas referee) should be sent to The Provost, University College London, Gower Street, London WC1E 6BT, to arrive by 1st December 1995.

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Cancer Epidemiologist

UTAH CANCER REGISTRY

The University of Utah and the Huntsman Cancer Institute invite applications for the Director of the Utah Cancer Registry. The Huntsman Cancer Institute is a new and rapidly expanding center of cancer research at the University of Utah. The Utah Cancer Registry plays a major role in facilitating cancer research activities of Huntsman Cancer Institute members and other cancer researchers in the intermountain area. The Utah Cancer Registry is one of the oldest cancer registries in the United States and has been part of the Surveillance Epidemiology and End Result (SEER) Program sponsored by the National Cancer Institute since 1973. The Director of the Utah Cancer Registry will assume programmatic and administrative responsibilities for the cancer registry and will provide leadership in developing and coordinating registry policies and procedures.

Candidates should have an M.D. with training in epidemiology or a doctoral degree in epidemiology or related scientific discipline and must qualify for an appointment at the Associate Professor level. The successful candidate must have a proven record of excellence in cancer research and management of research projects as demonstrated by cancer-related publications and ability to obtain research funding. We invite applications from individuals who understand issues related to gathering complex medical data and are able to communicate effectively with a wide variety of individuals within the scientific, medical, and lay communities. It is expected that the successful candidate will continue to develop a cancer-related research program within the Huntsman Cancer Institute.

To apply for this position, send a cover letter, a current CV, and names of individuals whom we may contact as a reference by February 29, 1996. Send applications to Marty Slattery, Ph.D., M.P.H., Department of Oncological Sciences/Division of Public Health Sciences, Room 5C334 SOM, University of Utah, Salt Lake City, Utah 84132. Additional information can be obtained by contacting Dr. Slattery at marty@possum.med.utah.edu.

HUNTSMAN CANCER INSTITUTE

AT THE UNIVERSITY OF UTAH

The University is an Equal Opportunity/Affirmative Action employer. Women, minority and international candidates are encouraged to apply.

MEETINGS

Optical Imaging of Gene Expression and Signaling in Living Cells

March 15 - 17, 1996

Conference Chairman: Steve Kay, *University of Virginia*

Keynote Address

Roger Tsien, *University of California, San Diego*
"Imaging Proteins and Gene Expression in Living Cells: GFP, beta-Lactamase, and Beyond"

Advanced Imaging Technology and Reporter Measurement

Masafumi Oshiro, *Hamamatsu Photonics USA*
Ted Salmon, *University of North Carolina*
Scott Fraser, *California Institute of Technology*
Keith Wood, *Promega Corporation*

Luciferase Applications

Jeremy Tavare, *University of Bristol, U.K.*
Stephen Frawley, *Medical University of South Carolina*
Andrew Millar, *University of Warwick, U.K.*
Lionel Jaffe, *Woods Hole Marine Biological Laboratory*

Applications of Green Fluorescent Protein and its Derivatives

Tulle Hazelrigg, *Columbia University*
Jim Haseloff, *Medical Research Council, U.K.*
Pamela Silver, *Harvard Medical School*

Abstracts may be submitted for poster presentation. The abstract deadline is January 15, 1996. *Registration fee*, including all meals and accommodation: Students $295, all others $385

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ASSISTANT/ASSOCIATE PROFESSOR
Biochemistry/Molecular Biology of Cancer

The Department of Biochemistry and Molecular Biology and Center of Excellence in Cancer Treatment, Research and Education (the Cancer Center) at the Louisiana State University Medical Center invite applications for a tenure-track faculty position. The Department and Center currently have strengths in prokaryotic and eukaryotic genetics, transcription and chromatin structure, translation and cell growth control, protein and biophysical chemistry, and signal transduction. Applicants with interests in the molecular biology of cancer research are particularly encouraged to apply. Applicants should have a doctoral degree, postdoctoral research experience and a commitment to excellence in teaching and research. The successful applicant should develop a strong, extramurally funded research program and contribute to the teaching of graduate and medical students. The Department and Center will assist with technical support and start-up funds. Send a curriculum vitae, statement of current and future research directions, and names of three references to: **Robert E. Rhoads, Ph.D., Professor and Head, Department of Biochemistry and Molecular Biology, LSU Medical Center, 1501 Kings Highway, Shreveport, LA 71130-3932, U.S.A.** *An equal opportunity employer.*

POSITION ANNOUNCEMENT

RANK AND TITLE: Professor and Head, Department of Animal Sciences, College of Agricultural, Consumer and Environmental Sciences, University of Illinois at Urbana-Champaign

DEPARTMENTAL DESCRIPTION:
The Department of Animal Sciences is administered by a Head, and includes 44 faculty involved in research, teaching and extension activities. Complete curricula are offered to 400 undergraduate majors and 100 graduate students in M.S. and Ph.D. programs in various specializations.

QUALIFICATIONS:
Candidate must hold a Ph.D. in one discipline of the animal sciences, be internationally recognized for contributions in research, resident and/or extension education, and be tenurable at the level of Professor. Administrative experience and familiarity with the land-grant system are desirable. The candidate should have strong communication skills, the ability to work effectively with faculty, students, staff, administrators, and industry clientele, and demonstrated ability in attracting extramural funding.

SALARY: Competitive and commensurate with experience.

NOMINATIONS AND APPLICATIONS:
Nominations/Applications must be received by December 15, 1995 to ensure full consideration. Qualified women and minority candidates are encouraged to apply. Applications, including a statement of interest in headship, resume, and names, addresses and phone numbers of three references should be sent to:

Search Committee for Head, Department of Animal Sciences
c/o D.L. Chicoine Interim Dean
College of Agricultural, Consumer and Environmental Sciences
101 Mumford Hall/1301 West Gregory Drive
Urbana, IL 61801
Attn: Linda Pein
Telephone: 217-333-0460/Telefax: 217-244-2911

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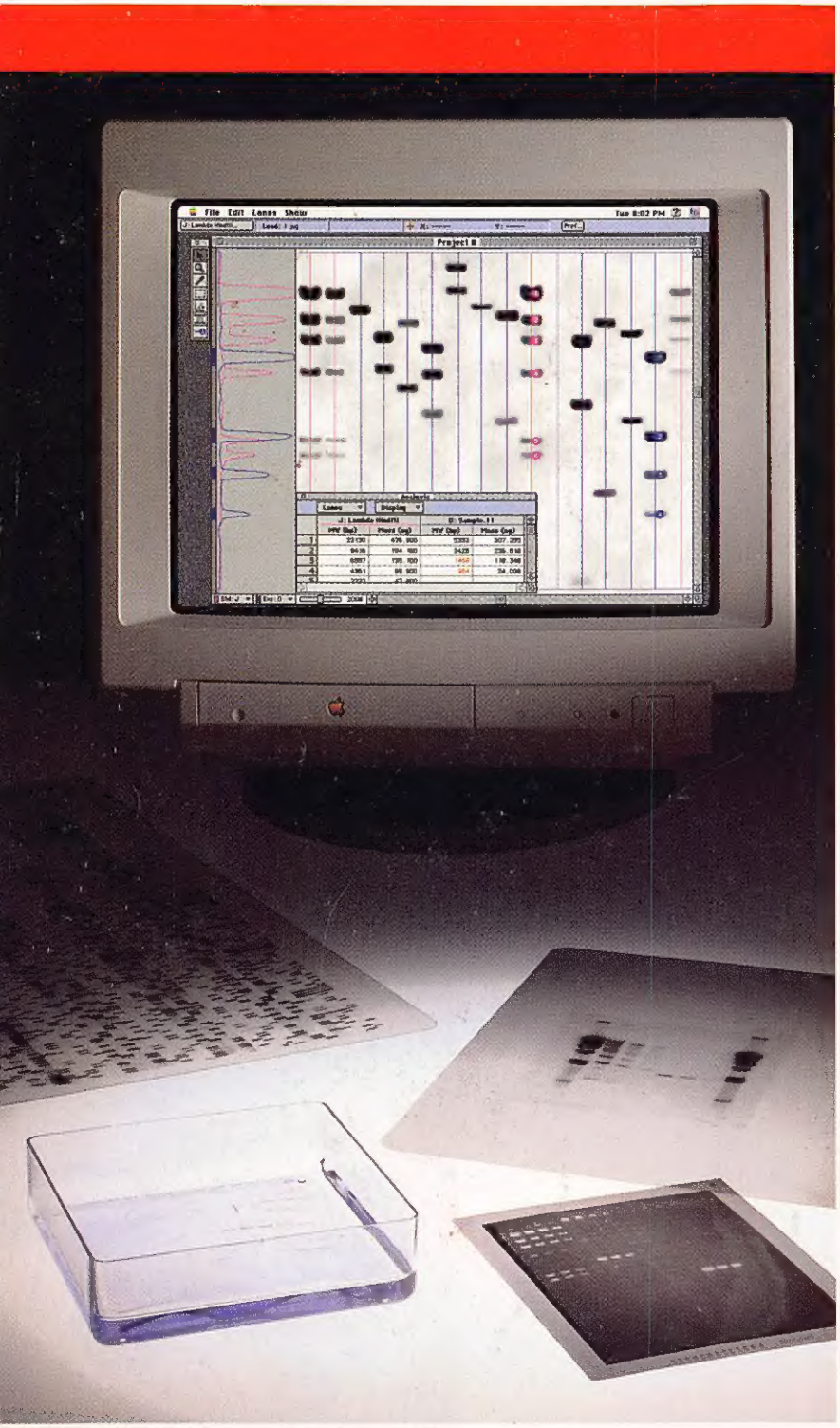

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